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A STUDY OF RENAL FUNCTION AND THE ASSOCIATED DISTURBANCE IN THE ACID-BASE EQUILIBRIUM OF THE BLOOD IN CERTAIN EXPERIMENTAL AND NATURALLY ACQUIRED NEPHROPATHIES *

WILLIAM DEB. MACNIDER, M.D.

CHAPEL HILL, N. C.

During the past fourteen years, the work in this laboratory has been confined largely to a study of different types of acute experimental nephropathy and of certain naturally acquired chronic nephropathies. Four hundred and forty-nine animals have been employed in the various series of experiments. In a large number of these animals some form of renal functional test was used. In 132 animals a functional study of the kidney was the primary object of the investigation. In this latter group of animals a variety of functional tests were made use of. With this large amount of experimental data available, a study has been undertaken of the function of the kidney in different types of nephropathic processes and of the association of changes in the acid-base equilibrium of the blood with variations in the functional and pathological response on the part of the kidney.

In the earlier series of experiments [1] which had as their object a study of the pathology of the acutely nephropathic kidney various substances were employed in an attempt to obtain some nephrotoxic agent which would produce in the kidney of the dog a reaction comparable to the acute nephropathy found in human material, in which the renal epithelium is primarily and more severely involved than is the vascular or interstitial tissue. The nephrotoxic agent which more nearly produces this type of reaction on the part of the kidney is uranium in the form of the nitrate. Christian and O'Hare [2] have shown that in the rabbit, uranium induces an early injury to the glomerular vessels. In

* From the Laboratory of Pharmacology of the University of North Carolina.

1. MacNider, William deB.: A Study of the Renal Epithelium in Various Types of Acute Experimental Nephritis and of the Relation which Exists Between the Epithelial Changes and the Total Output of Urine, J. Med. Res. **21**:79, 1912.

2. Christian, Henry A., and O'Hare, James P.: Glomerular Lesions in Acute Experimental (Uranium) Nephritis in Rabbits, J. Med. Res. **28**:227, 1913.

a later communication O'Hare [3] has pointed out the difficulty of inducing these changes in the glomerular vessels of the dog's kidney. From a large number of experiments on dogs I can confirm this observation. When uranium is given subcutaneously to the dog in small doses, properly spaced, the injury induced in the kidney is characterized by an acute tubular injury with an acute engorgement of the renal vessels, occasionally a small amount of exudate into the subcapsular space, but without degeneration in the vascular tissue. Substances such as the tartrates that have recently been employed by Underhill, Wells and Goldschmidt [4] as nephrotoxic agents are too selective in their affinity for the tubular epithelium and produce too rapidly an extensive necrosis of this tissue for them to be employed in experiments in which an attempt is made to duplicate experimentally the acute pathology found in certain of the acute nephropathies of human material. A second consideration which makes uranium an especially appropriate substance to use in such experimental studies, is that during the recovery of the kidney from the acute injury the processes of repair as was shown by Dickson [5] for the kidney of the guinea-pig, and more recently by MacNider [6] for the kidney of the dog, lead to the development of a chronic diffuse type of nephropathy. In this chronic nephropathy the glomeruli show both a capsular and intracapillary type of injury. The tubules show changes of repair in the regeneration of a flattened type of epithelium, which takes the place of the more specialized cells. Furthermore, functional studies in such nephropathic animals show these kidneys to resemble very closely the chronic diffuse nephropathy of human material, which originates as a reaction of repair to some diffusely acting nephrotoxic agent.

In the studies from this laboratory [7] on the chronic naturally acquired nephropathy of the dog, it was shown that the more common

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3. O'Hare, James P.: Acute Renal Lesions Produced by Uranium Nitrate in the Dog in Comparison with the Rabbit, Arch. Int. Med. **12**:61, 1913.

4. Underhill, Frank P., Wells, H. Gideon and Goldschmidt, Samuel: Tartrate Nephritis, with Special Reference to Some of the Conditions Under which It May Be Produced, J. Exper. Med. **18**:322, 1913.

5. Dickson, Ernest C.: A Report on the Experimental Production of Chronic Nephritis in Animals by the Use of Uranium Nitrate, Arch. Int. Med. **3**:375, 1909.

A Further Report on the Production of Experimental Chronic Nephritis in Animals by the Administration of Uranium Nitrate, Arch. Int. Med. **9**: 557, 1912.

6. MacNider, William deB.: A Functional and Pathological Study of the Chronic Nephropathy Induced in the Dog by Uranium Nitrate, J. Exper. M. **29**:513, 1919.

7. MacNider, William deB.: A Pathological Study of the Naturally Acquired Chronic Nephropathy of the Dog, Part I, J. Med. Res. **34**:177, 1916.

A Pathological and Physiological Study of the Naturally Nephropathic Kidney of the Dog Rendered Acutely Nephropathic by Uranium or by an Anesthetic, J. Med. Res. **34**:199, 1916.

type of injury was one in which the glomerulus was damaged out of proportion to the degree of tubular injury. This observation has been recently confirmed for human material by Stengel, Austin and Jonas.[8] Functional studies have been made in these naturally nephropathic animals and at a later period an acute tubular injury has been superimposed on the chronic pathology by employing uranium nitrate or mercuric chlorid. In these animals functional tests have again been employed to study the effect on function of the acute injury. By such a procedure it has been found possible to induce in the dog a pathologic condition and a functional response very similar to that obtained in human material in which an acute condition develops during the course of a chronic nephropathy and which not infrequently serves as a terminal change.

In the following study of renal functional tests, the observations will be confined to the functional response of the kidney in three types of injury. First, the function of the kidney will be studied in the earlier stages of an acute uranium intoxication when the pathology of the kidney is very largely confined to the tubular epithelium. In the second group of animals, observations will be made on the functional response of the kidney late in the uranium intoxication, when as a result of the processes of repair in both the tubules and the glomeruli a subacute or chronic nephropathy is being produced. The third group of animals is represented by dogs with a chronic naturally acquired nephropathy in which the renal injury is very largely confined to the glomeruli. At a later period in the study of these naturally nephropathic animals, renal functional tests have been employed after an acute process of epithelial degeneration had been superimposed on the chronic pathology by the use of uranium nitrate or mercuric chlorid.

TECHNIC OF EXPERIMENTS

All of the animals employed in this study, whether normal or naturally nephropathic, have been subjected to a routine type of study both before and during the course of the experiments. One hundred and five animals have been employed in the various groups of experiments. In Group I, thirty-two animals have been studied during the early stages of an acute uranium intoxication. Twenty-eight animals have been employed in Group II, representing the later stages in an acute uranium intoxication, in which the changes of repair lead to the development of a chronic nephropathy. Forty-five animals have been used in Group III. This group is composed of naturally nephropathic

8. Stengel, A., Austin, J. H., and Jonas, L.: A Comparison of the Functional Findings in a Series of Cases of Renal Disease, Arch. Int. Med. **21**: 313, 1918.

animals. In these animals an acute epithelial injury has been superimposed on the chronic naturally acquired pathology. All of the animales were kept in metabolism cages for from two to four days before any experimental procedure was commenced. During this period and after the commencement of the experiments, the animals were fed on bread containing a small amount of cooked meat. The animals received by stomach tube, once a day, 500 c.c. of water. Those animals which were used during the summer months were watered twice a day. The female dogs were catheterized either once or twice a day, and the urine mixed with the cage urine for analysis. All of the animals, except a certain number of the naturally nephropathic animals, which were given mercuric chlorid, at some stage of the experiment received subcutaneously 4 mg. per kilogram of uranium nitrate at one injection. Both before and after the commencement of the experiments the following observations have been made. The urine was subjected to the ordinary qualitative analysis. Quantitative determinations of albumin were made with both Esbach's and Tsuchiya's reagents. The output of acetone and diacetic acid was determined by the method of Folin [9] as modified by Hart.[10] The phenolsulphonephthalein elimination was determined for two hour periods according to the technic devised by Rowntree and Geraghty.[11] The centrifugalized urines were examined for casts. Blood urea determinations were made by the method of Marshal [12] as modified by Van Slyke and Cullen.[13] The estimations of blood creatinin were made by colorimetric determinations. A standard solution of creatinin in saturated picric acid was employed in preference to the standard potassium dichromate solution. The former solution is more accurate. In studying the changes in the acid-base equilibrium of the blood, the methods of Marriott [14] have been employed to ascertain the alkali reserve of the blood and the tension of alveolar air carbon dioxid.

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9. Folin, O.: On the Separate Determinations of Acetone and Diacetic Acid in Diabetic Urines, J. Biol. Chem. **3**:177, 1907.

10. Hart, T. S.: On the Quantitative Determination of Acetone in the Urine, J. Biol. Chem. **4**:477, 1908.

11. Rowntree, L. G., and Geraghty, J. T.: An Experimental and Clinical Study of the Functional Activity of the Kidneys by Means of Phenolsulphonephthalein, J. Pharmacol. & Exper. Therap. **1**:579, 1909.

12. Marshall, E. K., Jr.: A Rapid Clinical Method for the Estimation of Urea in Urine, J. Biol. Chem. **14**:283, 1913.

13. Van Slyke, D. D., and Cullen, G. E.: A Permanent Preparation of Urease, and Its Use in the Determination of Urea, J. Biol. Chem. **19**:211, 1914.

14. Marriott, W. McK.: A Method for the Determination of the Alkali Reserve of the Blood Plasma, Arch. Int. Med. **17**:840, 1916.

Marriott, W. McK.: The Determination of Alveolar Carbon Dioxid Tension by a Simple Method, J. A. M. A. **66**:1594 (May 20) 1916.

GROUP I. A FUNCTIONAL AND PATHOLOGIC STUDY OF THE KIDNEY IN THE EARLY STAGES OF AN ACUTE URANIUM NEPHROPATHY

In previous publications [15] the observation has been recorded that the toxicity of uranium for the dog is very definitely associated with the age of the animal. Regardless of the weight of the animal, uranium is more toxic for the older animals than for the young animals. This is shown in the rapidity of the development and the severity of the kidney injury. This factor, together with the dose of uranium and the duration of the intoxication, has to be taken into consideration in order to obtain a nephropathy typical for the early stages of a uranium intoxication. In this first group of acutely nephropathic animals, this factor of the age of dogs has been taken into consideration. The experiments as detailed in Tables 1, 2, 3 and 4, not only show the

TABLE 1.—GROUP I, EXPERIMENT 4. ACUTE URANIUM NEPHROPATHY

Animal: Age, 7 years; weight, 15.2 kg.

Day of Exp.	Water in 24 Hrs., C.c.	Uranium Nitrate per Kg.	Urine in 24 Hrs., C.c.	Albumin and Casts	Acetone, Mg. per 100 C.c.	Diacetic Acid, Mg. per 100 C.c.	Blood Urea, Mg. per 100 C.c	Blood Creatinin, Mg. per 100 C.c.	Phthalein 2 Hrs., per Cent.	R. p. H.	Carbon Dioxid Tension, Mm.
Normal	500	0	390	0	0	0	18	1.98	84	8.1	45
1	500	4 mg.	915	2 gm. Numerous casts	0	0	39	2.41	20	7.9	33
2	500	....	697	3.6 gm. Numerous casts	1.632	3.265	72	3.81	Trace	7.85	27
4	500	....	71	2 gm.	2.425	2.699	117	5.72	0	7.75	19
6	500	....	74	Trace	0	1.102	284	7.64	0	7.7	12

course of the intoxication in its early stages, but also serve to emphasize the importance of the age of the animal in determining the acuteness and severity of the intoxication. Thirty-two dogs were employed in this first group of experiments. The animals were kept in metabolism cages and normal observations made for two to four days. A one-day period of normal observations is included at the commencement of each table. After the period of normal observation the animals received one subcutaneous injection of 4 mg. per kilogram of uranium nitrate. All of the experiments were terminated on the sixth day of the intoxication. An anesthetic was not employed for this purpose. As has been previously shown,[16] the acutely nephropathic

15. MacNider, William deB.: A Consideration of the Relative Toxicity of Uranium Nitrate for Animals of Different Ages, J. Exper. Med. **26**:1, 1917. MacNider, William deB.: Concerning the Influence of the Age of an Organism in Maintaining Its Acid-Base Equilibrium, Science **46**:643, 1917.

16. MacNider, William deB.: The Effect of Different Anesthetics on the Pathology of the Kidney in Acute Uranium Nephritis, J. Med. Res. **27**:403, 1913.

kidney is extremely sensitive to the effect of the general anesthetics. If an anesthetic is employed in such an experiment the epithelial pathology of the kidney is grossly altered from the reaction induced by the nephrotoxic agent.

TABLE 2.—Group I, Experiment 18. Acute Uranium Nephropathy

Animal: Age, 13 months; weight, 12.58 kg.

Day of Exp.	Water in 24 Hrs., C.c.	Uranium Nitrate per Kg.	Urine in 24 Hrs., C.c.	Albumin and Casts	Acetone, Mg. per 100 C.c.	Diacetic Acid, Mg. per 100 C.c.	Blood Urea, Mg. per 100 C.c.	Blood Creatinin, Mg. per 100 C.c.	Phthalein 2 Hrs., per Cent.	R. p. H.	Carbon Dioxid Tension, Mm.
Normal	500	0	302	0	0	0	18	2.51	81	8.0	40
1	500	4 mg.	619	0.75 gm. No casts	8.865	2.790	28	2.58	56	7.95	38
2	500	0	300	2.7 gm. Casts present	3.825	1.816	59	3.41	0	7.9	34
4	500	0	412	0.5 gm.	3.614	1.532	81	5.71	0	7.85	53
6	500	0	281	Trace	3.021	1.502	146	5.79	0	7.85	30

TABLE 3.—Group I, Experiment 6. Acute Uranium Nephropathy

Animal: Age, 1 year; weight, 12.33 kg.

Day of Exp.	Water in 24 Hrs., C.c.	Uranium Nitrate per Kg.	Urine in 24 Hrs., C.c.	Albumin and Casts	Acetone, Mg. per 100 C.c.	Diacetic Acid, Mg. per 100 C.c.	Blood Urea, Mg. per 100 C.c.	Blood Creatinin, Mg. per 100 C.c.	Phthalein 2 Hrs., per Cent.	R. p. H.	Carbon Dioxid Tension, Mm.
Normal	500	0	500	0	0	0	17	1.85	83	8.05	41
1	500	4 mg.	485	0.4 gm. No casts	8.863	9.796	16	1.77	75	7.95	38
2	500	0	369	1.0 gm. Few casts	5.364	14.228	16	1.87	67	7.95	38
4	500	0	530	1.75 gm. Numerous casts	14.928	17.727	45	2.31	Trace	7.85	26
6	500	0	320	0.9 gm. Numerous casts	5.598	7.230	59	4.81	0	7.75	18

TABLE 4.—Group I, Experiment 20. Acute Uranium Nephropathy

Animal: Age, 11 months; weight, 6.8 kg.

Day of Exp.	Water in 24 Hrs., C.c.	Uranium Nitrate per Kg.	Urine in 24 Hrs., C.c.	Albumin and Casts	Acetone, Mg. per 100 C.c.	Diacetic Acid, Mg. per 100 C.c.	Blood Urea, Mg. per 100 C.c.	Blood Creatinin, Mg. per 100 C.c.	Phthalein 2 Hrs., per Cent.	R. p. H.	Carbon Dioxid Tension, Mm.
Normal	500	0	412	0	0	0	15	2.0	78	8.0	40
1	500	4 mg.	261	Trace No casts	8.630	3.032	15	2.0	60	7.95	37
2	500	0	375	0.6 gm. Few casts	28.923	39.186	22	2.31	51	7.9	33
4	500	0	329	1.4 gm. Few casts	4.009	8.086	48	3.82	30	7.9	33
6	500	0	230	Trace	5.134	4.721	80	4.71	0	7.75	20

A study of Tables 1, 2, 3 and 4 shows all of the animals to be normal prior to the use of uranium. The daily output of urine has varied from a minimum of 71 c.c. to a maximum of 915 c.c. The urine of all

of the animals was free from albumin and casts, and acetone and diacetic acid. The normal blood urea determinations varied from 15 to 18 mg. per hundred c.c. of blood. The blood creatinin determinations varied from 1.85 to 2.51 mg. per hundred c.c. of blood. The estimations have been very constant. In our experience, the blood creatinin for dogs has been somewhat higher than the findings of Myers and Lough [17] for human blood. The elimination of phenolsulphonephthalein in a two-hour period has varied between 69 and 84 per cent. The reserve alkali of the blood for the normal animals has varied between 8.0 to 8.1. The tension of alveolar air carbon dioxid in the different animals has shown a close correlation with the determinations of the alkali reserve. The tension of carbon dioxid in alveolar air has varied between 40 to 45 mm. of mercury. In two of the older animals of this series there was a lack of correlation between the determinations of the alkali reserve of the blood and the tension of carbon dioxid in alveolar air. At necropsy both of these dogs showed an advanced stage of pulmonary emphysema.

Following the commencement of the uranium intoxication, the effect on the daily output of urine is variable. In some of the animals there occurs an initial polyuria. This was the case in the animal of Experiment 4, Table 1. The output of urine increased from 390 c.c. on the day prior to the uranium to 915 c.c. on the first day of the uranium intoxication. In other animals, as is shown in the tables, the daily output of urine remains unchanged, or a slight reduction occurs. In all of the animals of this group albumin appeared in the urine within the first twenty-four hours. At this very early stage of the kidney injury, the association of casts with the albumin was variable. In those animals in which only a trace of albumin was found in the urine casts have frequently not been present. In those animals in which 1 gm. or more of albumin appeared in the urine casts have invariably been present. In animals which have been killed at this very early stage of the nephropathy in which the urine showed a trace of albumin but no casts, the pathology of the kidney has consisted in an acute engorgement of the glomerular tufts and no epithelial degeneration. In those animals that have shown both an albuminuria and casts, the renal epithelium has shown an early degeneration. From this observation it would appear that cast formation is more definitely associated with epithelial injury than it is with the contact of blood albumin with the acid environment of the kidney. A study of the four tables of experiments representing this group of animals shows the old animals of the group to have a higher percentage of albumin in the urine on the

17. Myers, Victor C., and Lough, Walter G.: Creatinin of the Blood in Nephritis. Its Diagnostic Value, Arch. Int. Med. **16**:536, 1915.

first and subsequent days of the experiments than do the young animals. This has been a very constant observation, and was the first point to call attention to the difference in the response of animals of different ages to a constant quantity of uranium. As will be noted, with the appearance of albumin in the urine there also occurs a reduction in the reserve alkali of the blood, a decrease in the tension of alveolar air carbon dioxid, and a sharp reduction in the elimination of phenolsulphonephthalein. In our long series of experiments there has been a constant association between the depletion of the blood of its alkali reserve from the use of uranium and the appearance of albumin in the urine. It is difficult to say that the reduction in the reserve alkali of the blood is the cause of the albuminuria. It can, however, be stated that with the reduction in the alkali reserve of the blood which is effected by uranium there is an associated albuminuria. In a recent research by Karsner [18] on the toxic effect of uranium in dogs he has confirmed the occurrence of a depletion of the alkali reserve of the blood with the beginning of the kidney injury. In a certain number of his animals the albuminuria developed prior to the reduction of the alkali reserve of the blood. This has not occurred in our studies with a large number of animals. We have found a constant association between the reduction in the reserve alkali of the blood and the appearance of albumin in a specimen of catheterized urine obtained at the same time that the alkali reserve determination was made.

A further study of the tables of experiments of this group of acutely nephropathic animals shows that not only is the output of albumin in the urine greater in the older animals, but the reduction in the elimination of phenolsulphonephthalein, the depletion of the reserve alkali of the blood, and the decrease in the tension of alveolar air carbon dioxid, are all more sharply affected than is the case with the younger animals of the group. The animal of Experiment 4, Table 1, 7 years old, on the first day of the uranium intoxication showed an albuminuria of 2 gm. per liter, a reduction in the output of phenolsulphonephthalein from a normal of 84 per cent. to 20 per cent., a depletion in the reserve alkali of the blood from a normal of 8.1 to 7.9 and a reduction of the carbon dioxid tension of alveolar air from 45 to 33 mm. The young animal of Group 1, Table 4, Experiment 20, 11 months old, showed during the first day of the uranium intoxication only a trace of albumin in the urine. The phenolsulphonephthalein elimination was only reduced from a normal of 78 per cent. to 60 per

18. Karsner, Howard T., Reimann, Stanley P., and Brooks, S. C.: Studies of Uranium Poisoning. IV. The Relation of Acid Intoxication to Nephritis, J. Med. Res. **39**:177, 1918.

cent., the reserve alkali of the blood was reduced from 8.0 to 7.95, and the tension of carbon dioxid in alveolar air was reduced from 40 to 33 mm.

A further study of the albuminuria during these experiments shows that as the severity of the kidney injury progresses, as indicated by a continuous reduction in the output of phenolsulphonephthalein, there occurs a decrease in the amount of albumin in the urine. Associated with the diminishing ability of the kidney to secrete phenolsulphonephthalein, there occurs a progressive reduction in the alkali reserve of the blood. There is a definite association between the severity of the kidney injury as shown by the phenolsulphonephthalein elimination and the depletion of the blood of its alkali reserve. There is, however, no correlation between the elimination of this dye and the disturbance in the acid-base equilibrium of the blood with the quantitative output of albumin in the urine. As will be seen in Experiment 4, on the second day of the uranium intoxication the urine showed 3.6 gm. of albumin per liter, the phenolsulphonephthalein elimination for two hours was only a trace and the alkali reserve of the blood showed a depletion to 7.85. On the sixth day of the experiment, with the total output of urine reduced to 74 c.c., the urine contained only a trace of albumin. The phenolsulphonephthalein output was negative, the reserve alkali of the blood was reduced to 7.7 and the tension of carbon dioxid in alveolar air was reduced to 12 mm. These experiments indicate very clearly that determinations of the quantitative output of albumin in the urine may give an entirely erroneous conception of the severity of the kidney injury. Each day during the course of these experiments at the same time that the previously mentioned observations were made, examinations of the blood were undertaken for evidence of kidney injury as indicated by a retention of blood urea and creatinin.

A study of the four tables of experiments in this group of animals shows that a retention of both urea and creatinin occurs not later than the second day of the acute kidney injury. Table 1, Experiment 4, which represents the older animals of the group, shows a retention of urea and creatinin on the first day of the experiment, while in the younger animals as indicated by Table 4, Experiment 20, there is no retention of these substances as early as the first day of the experiment, and not until the fourth day is the retention marked.

The various tables of experiments also show a definite correlation between the severity of the kidney injury as indicated by the elimination of phenolsulphonephthalein and the degree of depletion of the blood of its alkaline reserve with a retention of both urea and creatinin. In the old animal of Experiment 4, during the first day of the kidney

injury from uranium, the elimination of phenolsulphonephthalein was reduced from a normal of 84 per cent. to 20 per cent., and the alkali reserve of the blood was depleted from 8.1 to 7.9. During this period a retention of blood urea occurred from a normal of 18 mg. per hundred c.c. to 59 mg. The blood creatinin showed a retention from a normal of 1.98 mg. to 2.41 mg. In the case of the younger animal of Table 4, Experiment 20, with a less severe kidney injury, the same evidence of diminished renal function was shown, but the reduction in renal function developed more gradually. In this animal, on the first day of the renal injury, the phenolsulphonephthalein elimination was only reduced from a normal of 78 per cent. to 60 per cent., and the reserve alkali was only reduced from 8.0 to 7.95. During this first day there was no retention of either blood urea or creatinin. As the kidney injury progressed, as was shown by a further reduction in the elimination of phenolsulphonephthalein, there occurred both a retention of blood urea and creatinin. On the fourth day of this experiment, the elimination of phenolsulphonephthalein was 30 per cent., the blood urea retention was 80 mg. per hundred c.c., and the creatinin retention 4.91 mg.

From these observations it is clear that the earliest evidence of renal injury consists in a reduction in the output of phenolsulphonephthalein and the appearance of albumin in the urine. After a certain grade of renal injury is established there then occurs a retention of both urea and creatinin. During the remaining days of the experiments there has occurred with a progressive decrease in the ability of the kidney to eliminate phenolsulphonephthalein an increase in the retention of blood urea and creatinin. In the animal of Experiment 1, with a very severe kidney injury there was a retention of blood urea on the sixth day of the experiment of 284 mg., and a creatinin retention of 7.64 mg. In the animal of Experiment 20, with a less severe kidney injury, the blood urea retention was 80 mg., and the creatinin retention 4.71 mg.

During the course of these experiments determinations have been made of the time of appearance and quantitative output in the urine of both acetone and diacetic acid. These observations have been made in connection with changes in the alkali reserve of the blood and with the disturbance in renal function. A study of the table of experiments shows that the appearance of acetone and diacetic acid in the urine may or may not occur with the initial reduction in the alkali reserve of the blood. In Experiment 4, Table 1, the alkali reserve of the blood was reduced during the first day of the experiment from 8.1 to 7.9. The urine was free from both acetone and diacetic acid. In other animals of this group, Tables 2, 3 and 4, with a reduction in the alkali reserve, there also appeared in the urine both acetone and diacetic acid.

During the remaining days of the experiments with a progressive depletion in the alkali reserve, the output of acetone and diacetic acid in the urine not only failed to show an increase, but the output of both of these bodies was finally diminished.

A study of the tables from the standpoint of the influence of the degree of renal injury on the quantitative output of these bodies in the urine, shows that when the kidney is severely injured, as indicated by a negative elimination of phenolsulphonephthalein and a marked retention of blood urea and creatinin, that the output of acetone and diacetic acid is reduced and also that the reserve alkali of the blood shows a further depletion. It would appear from these observations that quantitative determinations of ketone bodies in the urine is in no sense a true expression of the disturbance in the acid-base equilibrium of the blood. The observations would furthermore indicate that with the development of a sufficient degree of renal injury the elimination of these bodies is interfered with and their retention may explain the severe depletion in the alkali reserve of the blood which is associated with some types of severe kidney injury.

REPORT ON MICROSCOPIC PATHOLOGY

All of the experiments included in Group 1 were terminated on the sixth day with the object of ascertaining the pathology of the kidney in a large number of animals that were showing the same type of functional response. Kidney tissue was at once removed and fixed in a 10 per cent. solution of liquor formaldehyd, Zenker's fluid and in a corrosive-acetic solution. Frozen sections were made from the liquor formaldehyd fixed tissue and stained for fat with Scharlach R. Sections from the Zenker and corrosive-acetic fixed tissue were stained with hematoxylin and eosin.

The histologic study of this tissue from the thirty-two acutely nephropathic animals has shown the same type of renal injury. The glomerular vessels have been engorged with blood generally obliterating the subcapsular space. In seven of the animals an exudate of amorphous material, with no definite fibrin, was found in the capsular space and slightly compressed the capillary tufts. The exudates have shown a few red blood cells. The intertubular vessels showed a similar engorgement, but no exudate has been observed between the tubules. The vascular tissue of the kidney has shown no other evidence of injury. Fat has not been demonstrated in the glomerular or intertubular vessels. No cellular reaction has been observed in the glomerular vessels. The vascular pathology in all of the animals has consisted in an acute engorgement of the vessels which has varied in severity.

The absence of thrombi in the vessels and the lack of any cellular reaction would argue against the existence of an injury of a degenerative character.

The pathologic response on the part of the kidney, which has been constant in type but which has varied in severity, has consisted in an acute injury to the renal epithelium. Two types of degenerative changes have occurred. The epithelium of the convoluted tubules has

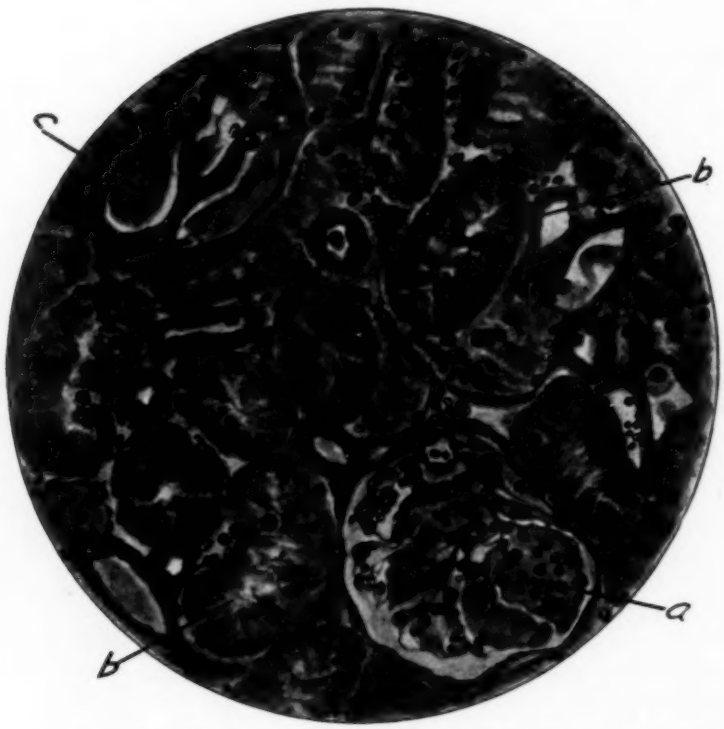

Fig. 1.—Camera lucida drawing, Leitz oc. 2, obj. 6. The figure is from the kidney of the animal of Experiment 4, Table 1, Group I. Following one injection of 4 mg. per kilogram of uranium, the animal developed a severe tubular nephropathy. The output of urine was greatly reduced. The alkali reserve was reduced to 7.7. The phenolsulphonephthalein elimination was negative. There was a retention of both blood urea and creatinin. At A is shown a normal glomerulus. At B are shown the convoluted tubules severely swollen with a beginning necrosis. At C are junctional tubules containing casts.

shown the severest grade of injury. This injury has consisted in an acute swelling and vacuolation of the cells, which is followed by necrosis. Fat occurs in the degenerating epithelium in the form of

numerous fine, dustlike granules. The epithelium of the ascending limb of Henle's loop has shown some swelling but no vacuolation. The cells of this type of epithelium contain a large amount of stainable fat. The fat in this epithelium appears in the form of large droplets which frequently fuse together and obliterate the structure of the cell (Fig. 1).

SUMMARY OF THE ACUTELY NEPHROPATHIC ANIMALS OF GROUP I.

1. The injury induced in the kidney of the dog by one subcutaneous injection of 4 mg. of uranium nitrate per kilogram and extending over a period of six days is essentially a tubular nephropathy. The tubular injury is very largely confined to the convoluted tubule epithelium and to the epithelium of the ascending limb of Henle's loop.

2. The earliest functional disturbance indicative of the development of this injury has consisted in the appearance of albumin in the urine and a reduction in the elimination of phenolsulphonephthalein.

3. Coincident with the appearance of albumin in the urine and the reduction in the elimination of phenolsulphonephthalein there occurs a disturbance in the acid-base equilibrium of the blood, which is indicated by a reduction in the alkali reserve of the blood and a decrease in the tension of alveolar air carbon dioxid.

4. In such acute tubular nephropathies there exists a correlation between the degree of depletion of the alkali reserve of the blood and the elimination of phenolsulphonephthalein.

5. The quantitative output of albumin in the urine is no index of the severity of the renal injury. The output of albumin in the urine may progressively decrease, while the elimination of phenolsulphonephthalein is reduced to a negative quantity.

6. Associated with the reduction in the alkali reserve of the blood, the appearance in the urine of ketone bodies is variable. The quantitative output of these bodies in the urine is not a quantitative expression of the degree of disturbance in the acid-base equilibrium of the blood. With the development of a severe grade of renal injury as indicated by the elimination of phenolsulphonephthalein, the output in the urine of both acetone and diacetic acid is reduced. Associated with this reduced elimination of these bodies there occurs a further disturbance in the acid-base equilibrium of the blood which is indicated by a further depletion in the alkali reserve and a decrease in the tension of carbon dioxid in alveolar air.

7. After the establishment of the renal injury, as indicated by the appearance of albumin in the urine and a reduction in the elimination of phenolsulphonephthalein, there occurs a retention of blood urea and creatinin.

8. The percentage retention of these substances increases with the severity of the injury to the tubular epithelium.

9. The rapidity with which the renal injury develops, the severity of the injury, and the degree of the functional disturbance as indicated by the various functional tests is strikingly influenced by the age of the animal.

10. The older animals show a more severe type of nephropathy and an earlier and more marked functional disturbance than do the young animals.

GROUP II. RECOVERY EXPERIMENTS FROM AN ACUTE RENAL INJURY DUE TO URANIUM WITH THE PRODUCTION OF A CHRONIC TYPE OF NEPHROPATHY

Twenty-eight dogs have been employed in this group of experiments. The animals have varied in age from 9 years and 2 months to 9 months. The acute renal injury has been produced in this group of animals in a manner similar to that for the first group. The animals were given subcutaneously one dose of uranium nitrate of 4 mg. per kilogram. The same general technic regarding food and water and the same tests for renal function and for ascertaining the degree of disturbance in the acid-base equilibrium of the blood have been employed in this group of animals as was used for the former group.

Depending on the age of the animals used in this second group of experiments, the animals may be divided into two classes, those animals which show a partial recovery from the acute renal injury and by the processes of repair in the kidney develop a chronic type of nephropathy, and those animals which are unable to further the processes of repair so that a chronic type of renal injury can be produced and so enable the animal to recover partially. The old animals of the group fall in this latter class, which fail to recover. We have been unable to obtain such recovery experiments in animals more than 2 years of age. The younger animals of the group have shown various degrees of recovery. During the course of the uranium intoxication in these animals, the experiments have been terminated at various periods in order to observe the pathologic changes in the kidney at various stages in the functional recovery of the organ from the acute nephropathy. The earliest period for the termination of an experiment has been on the tenth day. The longest period for the duration of an experiment has been ninety-one days. For the sake of reducing the length of the tables in recording the course of these experiments, observations have only been recorded on every other day or in some instances on every fifth day of the experiment. Routine examinations were made, however, every day for the duration of the intoxication.

In order to demonstrate the influence of the age of the animal on the course of the recovery three experiments have been selected from animals of different age periods. Experiment 24, Group 2, Table 5 was carried out in an animal 4 years and 2 months old. Experiment 31,

TABLE 5.—GROUP II, EXPERIMENT 24. RECOVERY EXPERIMENT FROM URANIUM WITH THE PRODUCTION OF A CHRONIC NEPHROPATHY

Animal: Age, 4 years, 2 months; weight, 15.2 kg.

Day of Exp.	Water in 24 Hrs., C.c.	Uranium Nitrate per Kg.	Urine in 24 Hrs., C.c.	Albumin and Casts	Acetone, Mg. per 100 C.c.	Diacetic Acid, Mg. per 100 C.c.	Blood Urea, Mg. per 100 C.c.	Blood Creatinin, Mg. per 100 C.c.	Phthalein 2 Hrs., per Cent.	R. p. H.	Carbon Dioxid Tension, Mm.
Normal	500	0	360	0	0	0	18	2.3	84	8.1	45
1	500	4 mg.	915	2 gm. Casts	0	0	59	3.0	29	7.85	35
2	500	0	697	3.6 gm. Casts	1.632	3.265	72	4.31	Trace	7.9	30
4	500	0	587	4 gm. Numerous casts	3.498	2.352	120	4.52	0	7.8	20
6	500	0	184	4 gm. Numerous casts	2.425	2 009	121	5.08	0	7.75	20
8	500	0	100	2 gm. Numerous casts	Not made	Not made	134	5.26	0	7.75	20
10	500	0	0	Not made	Not made	Not made	284	6.18	No urine	7.7	12

TABLE 6.—GROUP II, EXPERIMENT 3. RECOVERY EXPERIMENT FROM URANIUM WITH THE PRODUCTION OF A CHRONIC NEPHROPATHY

Animal: Age, 1 year, 4 months; weight, 15.9 kg.

Day of Exp.	Water in 24 Hrs., C.c.	Uranium Nitrate per Kg.	Urine in 24 Hrs., C.c.	Albumin and Casts	Acetone, Mg. per 100 C.c.	Diacetic Acid, Mg. per 100 C.c.	Blood Urea, Mg. per 100 C.c.	Blood Creatinin, Mg. per 100 C.c.	Phthalein 2 Hrs., per Cent.	R. p. H.	Carbon Dioxid Tension, Mm.
Normal	500	0	340	0	0	0	12	2.0	73	8.1	43
1	500	4 mg.	626	1.2 gm. Few casts	0	0	16	2.0	61	7.95	38
2	500	0	481	1.1 gm. Casts	1.208	2.755	24	2.0	45	7.95	38
4	500	0	210	0.6 gm. Casts	5.059	7.397	48	2.31	21	7.9	30
6	500	0	230	0.2 gm. Casts	5.905	2.755	70	2.91	Trace	7.9	30
10	500	0	381	0.2 gm. Casts	3.008	4.639	100	3.41	0	7.85	24
14	500	0	503	Trace	2.417	3.442	200	4.281	0	7.85	25
18	500	0	600	0	2.650	4.390	206	4.28	Trace	7.85	23
22	500	0	700	0	2.707	4.303	208	4.28	Trace	7.85	25
26	500	0	794	0	10.782	6.865	218	4.30	10	7.9	32
30	500	0	628	0	9.285	13.581	180	4.32	10	7.9	33
34	500	0	623	Trace	12.681	16.342	170	4.18	15	7.95	35
38	500	0	346	Few casts 0	Trace	1.819	148	3.61	15	7.95	35

Group 2, Table 6, in an animal 1 year and 4 months old, while Experiment 8, Group 2, Table 7 was conducted in an animal 9 months old.

A study of the tables of experiments, which are representative of this group of animals, shows a response on the part of the animals

similar in type to that described in detail for the animals of Group 1. It will be noted, as was the case for the former group, that the functional disturbance of the kidney and the change in the acid-base equilibrium of the blood is more rapidly produced and is of greater severity in the old animal of Experiment 24 than it is in the young animal of Experiment 8. A study of Experiment 24, Table 5, which represents the older group of animals, shows that during the first day of the uranium intoxication there occurred a sudden reduction in the reserve alkali of the blood from 8.1 to 7.95, a reduction in the tension of carbon

TABLE 7.—Group II, Experiment 8. Recovery Experiment from Uranium with the Production of a Chronic Nephropathy

Animal: Age, 9 months; weight, 13.42 kg.

Day of Exp.	Water in 24 Hrs., C.c.	Uranium Nitrate per Kg.	Urine in 24 Hrs., C.c.	Albumin and Casts	Acetone, Mg. per 100 C.c.	Diacetic Acid, Mg. per 100 C.c.	Blood Urea, Mg. per 100 C.c.	Blood Creatinin, Mg. per 100 C.c.	Phthalein 2 Hrs., per Cent.	R. p. H.	Carbon Dioxid Tension, Mm.
Normal	500	..	605	0	0	0	18	2.03	84	8.1	45
1	500	4 mg.	619	Trace	8.863	2.799	28	2.03	56	8.0	40
2	500	..	300	2.2 gm. Casts	3.416	4.315	59	2.71	Trace	7.95	34
4	500	..	434	9.6 gm. Casts	12.631	13.0	62	2.70	Trace	7.95	35
6	500	..	284	0.4 gm. Casts	9.436	21.321	77	2.70	Trace	7.95	35
8	500	..	475	Trace Few casts	10.796	9.433	177	3.14	0	7.85	23
10	500	..	198	0	9.430	0.993	180	3.14	0	7.75	20
12	500	..	397	0	6.904	8.162	297	4.75	0	7.75	18
16	500	..	511	Trace Casts	6.064	12.498	258	5.31	14	7.8	20
20	500	..	643	Trace Casts	11.942	23.831	183	5.25	24	7.85	25
25	500	..	612	Trace Casts	14.713	21.931	108	4.64	24	7.9	30
30	500	..	450	Trace Casts	16.431	20.820	73	4.50	29	7.9	30
35	500	..	561	Trace Casts	6.994	6.960	56	4.18	35	7.9	30
37	500	..	381	0.25 gm. Casts	8.641	7.982	45	3.75	37	7.95	30
40	500	..	523	0.7 gm. Casts	6.064	10.293	51	3.75	37	7.95	30
45	500	..	525	0.2 gm. Casts	6.064	3.245	61	3.25	36	8.0	40
50	500	..	431	Trace Casts	8.132	4.176	43	2.78	38	8.0	40
60	500	..	732	Trace Casts	7.624	8.315	40	2.76	38	8.0	40

dioxid in alveolar air from 45 to 35 mm., a decrease in the elimination of phenolsulphonephthalein from 84 per cent. to 20 per cent., and the appearance of both albumin and casts in the urine. In these old and highly susceptible animals there furthermore occurs during the first day of the experiment a retention of both blood urea and creatinin. The blood urea retention is proportionately larger than is the retention of creatinin. In this susceptible group of old animals there occurs a rapid depletion in the reserve alkali of the blood, the elimination of phenolsulphonephthalein is reduced to zero in four to six days, and

there occurs a progressive retention of blood urea and creatinin. In Experiment 24 the output of urine was decidedly reduced by the sixth day, and by the tenth day the animal was anuric. On this day the reserve alkali of the blood had been depleted to 7.7 and the tension of alveolar air carbon dioxid had been reduced to 12 mm. The animal was semicomatose with an atypical type of air hunger breathing. Blood urea showed a retention of 284 mg., as compared with the normal of 18 mg. and blood creatinin a retention of 6.18 mg. in contrast with the normal of 2.3 mg.

The pathologic study of the kidneys of these old animals that have shown no attempt to reestablish a normal acid-base equilibrium of the blood and, furthermore, no indication of a restoration of renal function, have failed to show any evidence of reparative changes of a constructive character. Mitotic figures have not been observed in the damaged renal epithelium, and no other evidence of regeneration has been obtained. The cells of the convoluted tubules in the uranium nephropathies of such duration are largely necrotic. Vacuolation and the associated swelling are marked, so that in many of the tubules the lumen has become obliterated by the edematous cells. These cells contain a large amount of fat. The cells of the ascending limb of Henle's loop show a similar type of reaction. The necrosis is not so extensive. In such a prolonged injury from uranium the vascular tissue of the kidney becomes involved in the reaction. In addition to the acute engorgement of the glomerular vessels which was noted as the essential change in the vascular pathology during the early stage of a uranium nephropathy, in these injuries of longer duration thrombi have been observed in the tuft of capillaries. An exudate containing fibrin has been noted in the subcapsular space adhering to the capsular epithelium. In such an exudate as well as in the thrombi of the capillaries, endothelial leukocytes have been active. The capsular epithelium is swollen and in such injured cells mitotic figures have been observed. The acute nephropathy of ten days or more duration in these old and susceptible animals is not only characterized by an injury to the epithelium of the tubules, but by an injury to the glomeruli. Regenerative changes do not occur in the tubules of such animals and there is no attempt at a restoration of renal function.

A study of Experiments 3 and 8, Tables 6 and 7, which represent typical experiments in the younger animals of the group, show a similar type of response from uranium as did the older animals. The intoxication in the younger animals, however, as is indicated by the disturbance in the acid-base equilibrium of the blood and by the various tests for renal function, is not so abrupt in its onset and the disturbance is not so marked early in the experiments. During the period of the acute kidney injury, before there occurs any evidence of a come-back

on the part of the kidney, a marked depletion in the alkali reserve of the blood develops and renal function is seriously interfered with. In Experiment 3, by the tenth day of the uranium intoxication the reserve alkali of the blood had been depleted to 7.85, the output of phenolsulphonephthalein was reduced to zero, there was a retention of blood creatinin of 3.41 mg. and of blood urea of 100 mg. With this degree of disturbance in the acid-base equilibrium of the blood and in renal function the urine showed only 0.2 gm. of albumin per liter. At this period of the experiment the urine contained both acetone and diacetic acid. It will be noted, however, that these bodies were present in less amount than they were early in the experiment when renal function was not so severely interfered with, and at which time the kidney was still able to secrete these bodies.

In Experiment 8, Table 7, the same type of injury is induced. In this animal, on the twelfth day of the uranium intoxication, the reserve alkali of the blood was depleted to 7.75, and the tension of alveolar air carbon dioxid was reduced to 18 mm. Prior to this on the eighth day of the experiment the elimination of phenolsulphonephthalein had reached the zero point and remained negative for four days. On the twelfth day the injury to the kidney was furthermore shown by a creatinin retention of 4.75 mg. and a retention of blood urea of 297 mg. Both acetone and diacetic acid were present in the urine, but present in a smaller percentage than was the case earlier in the experiment when all of the tests showed a higher grade of renal function. A continuation of the study of Experiments 3 and 8, which were conducted in young dogs, shows that from the fourteenth to the eighteenth days of the nephropathy the injury to the kidney has reached its height. From this period to the termination of the experiments there is an attempt on the part of the kidney to reestablish some degree of function.

The pathologic studies have shown that commencing with this period of the experiment there is histological evidence of changes of repair and regeneration of renal epithelium. The first evidence of an improvement in renal function has consisted in the reappearance of phenolsulphonephthalein in the urine. In Experiment 3 the dye first appeared on the eighteenth day, while in Experiment 8 the appearance was first noted on the fourteenth day. For the first two to four days, after the reappearance of phenolsulphonephthalein, the output has been so small as to render difficult its determination. Following this period the output has increased so that in from four to eight days the elimination has increased from 10 to 14 per cent. With the reappearance of phenolsulphonephthalein in the urine no immediate change takes place in the retention of urea and creatinin, and neither is there any immediate change in the degree of depletion of the alkali reserve of the blood. In from four to six days after the first appearance of

phenolsulphonephthalein in the urine, at which time the animals were eliminating from 10 to 24 per cent. of the dye, there has occurred a beginning increase in the alkali reserve of the blood.

At the same time that this change takes place in the blood, there has occurred an increase in the output in the urine of both acetone and diacetic acid. This observation would tend to show that a part of the disturbance in the acid-base equilibrium of the blood induced by uranium is due to a retention of acid bodies. Furthermore, that with an improvement in renal function as was indicated by the elimination of phenolsulphonephthalein, certain bodies which the kidney has been unable to secrete, for instance acetone and diacetic acid, are now eliminated, and with their removal there occurs a beginning reestablishment of the acid-base equilibrium of the blood. Experiment 8 very clearly illustrates these changes. For four days after the kidneys were eliminating from 14 to 24 per cent. of phenolsulphonephthalein there was no change in the alkali reserve of the blood. At the end of this period the reserve alkali increased from 7.8 to 7.9 and the tension of carbon dioxid in alveolar air increased from 20 to 30 mm. During this stage of the experiment the elimination of acetone increased from 6.064 to 14.713 mg. per hundred c.c. of urine, and the elimination of diacetic acid has increased from 12.498 to 21.931 mg. per hundred c.c.

A study of Experiments 3 and 8 furthermore shows that the threshold in the nephropathic kidney for the elimination of urea and creatinin is higher than it is for phenolsulphonephthalein. In Experiment 3 the retention of both urea and creatinin continued for four and six days, respectively, after the elimination of phenolsulphonephthalein had reached 10 per cent. Following this period there first occurred a decrease in the retention of blood urea and four days later the retention of creatinin began to decrease.

A further study of these experiments shows that following the commencement of the period of improvement in renal function there occurs a gradual increase in the elimination of phenolsulphonephthalein, a decrease in the retention of urea and creatinin, and an increase in the reserve alkali of the blood to such an extent that the acid-base equilibrium of the blood is either restored or brought near the point of normality. In nine of the animals used in the group the functional capacity of the kidney underwent such an improvement during the period of recovery that between the eightieth and ninetieth days of the experiment the acid-base equilibrium of the blood had been restored to the normal. In the remaining animals of the group there occurred an increase in the reserve alkali, but the normal acid-base equilibrium was not reestablished.

The period of recovery of the kidney from the acute nephropathy with a partial return of renal function during the development of the

chronic nephropathy is well shown in Experiment 8, Table 7. At the height of the acute injury, on the twelfth day of the experiment, the reserve alkali was reduced to 7.75. In a two hour period there was no elimination of phenolsulphonephthalein. The blood showed a retention of 297 mg. of urea and a creatinin retention of 4.75 mg. At the termination of the experiment on the sixtieth day the reserve alkali had increased to 8.0, and the tension of carbon dioxid in alveolar air to 40 mm. There was an elimination of phenolsulphonephthalein of 38 per cent. in two hours. The urea retention had been reduced to 49 mg. and the creatinin retention had decreased to 2.76 mg. A study of this experiment, and the following observation, shows even more clearly in those animals that are able to restore their acid-base equilibrium to the normal that the elimination of phenolsulphonephthalein does not show a correlation in quantitative output with the change towards the normal in the alkali reserve of the blood. The elimination of phenolsulphonephthalein was in no instance over 40 per cent. in the nine animals that succeeded in reestablishing their normal acid-base equilibrium. An animal with a severe grade of chronic nephropathy may have a normal alkali reserve of the blood and a normal tension of carbon dioxid in alveolar air. As will be shown later in Group III, such a finding is no expression of the stability of the acid-base equilibrium in a given animal. Determinations of the alkali reserve of the blood can in no sense be employed as an index of renal function or as an index of the ability of the kidney to withstand injury.

THE PATHOLOGY OF THE KIDNEY IN THOSE ANIMALS OF GROUP II THAT HAVE RECOVERED FROM THE ACUTE RENAL INJURY WITH THE DEVELOPMENT OF A CHRONIC NEPHROPATHY

The pathologic changes observed in the kidneys of this group of animals have depended on the stage of recovery at which the kidney tissue was obtained. The earliest evidence of repair in the kidney has been found between the eighth and fifteenth days of the intoxication. It will be recalled in this connection that the earliest indication of any functional restoration developed between the fourteenth and eighteenth day and consisted in the reappearance of phenolsulphonephthalein in the urine. The first evidence of repair in the kidney has consisted in a beginning regeneration of tubular epithelium. This first occurs in the terminal portion of the proximal convoluted tubule and in the upper portion of the descending limb of Henle's loop. The regeneration of cells occurs by a process of indirect cell division of the epithelium in those portions of the tubule that have not been too severely injured. The newly formed cells are large and vesicular with hyperchromatic

nuclei. Frequently the cell boundaries are not clearly differentiated. This gives rise to the formation in some tubules of a syncytial-like layer of epithelium which grows into the severely damaged convoluted tubule. At a later period the syncytial cytoplasmic mass differentiates into cells. At this early period of the renal injury there has been found an early cellular intertubular fibrosis. The glomerular vessels are invaded with fibroblasts and endothelial leukocytes and the capsular epithelium is hyperplastic.

In experiments that were terminated later in the period of recovery, between the twentieth and thirtieth day, the changes of repair have shown an increase. Many of the convoluted tubules are lined by the newly formed epithelium, which, however, has become changed in character. The cells which at first were large and vesicular have flattened out so that the tubules are lined by regenerated cells of a flattened type with a small amount of surrounding cytoplasm. The nuclei are large in proportion to the cytoplasm, and frequently project on the free margin of the cell. These cells are less specialized in structure than are the normal cells of the convoluted tubule. At this stage of repair in the kidney, the intertubular connective tissue has become more fibrous in character. In the glomeruli there is an early laying down of connective tissue which mats together the capillary loops and the walls of the capillaries become thickened. Between the capillary tufts and the capsule, the laying down of young connective tissue results in the formation of adhesions. The capsules show an increase in spindle-shaped cells and later become thickened. Around the glomeruli there is an early cellular periglomerular fibrosis.

A review of Experiments 3 and 8 indicates that at this stage of the experiments, between the twentieth and thirtieth days, an improvement in the functional response of the kidney occurs which is indicated by a further increase in the elimination of phenolsulphonephthalein, which may be as much as 29 per cent. There has occurred a decrease in the retention of blood urea and creatinin, an increase in the elimination of ketone bodies and an increase in the alkali reserve of the blood. The following observation is significant and of great importance that at this stage of the experiment when the tubular epithelium is being replaced, but when the glomeruli are being permanently damaged by the formation of fibrous tissue which obliterates the capillaries and causes a thickening of the capsule, there is a progressive increase in the functional capacity of the kidney. From this observation it appears only just to infer that the epithelial tissue of the kidney is of more functional value than is the vascular tissue.

The pathologic study of the kidney of animals late in the recovery from the acute renal injury when the chronic nephropathy has been definitely established shows a continuation of those processes of repair

previously described. The replacement of a flattened and atypical type of epithelium in the convoluted tubules continues. Many of the tubules show the original convoluted tubule epithelium in various stages of degeneration, while other tubules show a regeneration of this type of cell. Such damaged cells show much stainable fat. The intertubular connective tissue, though not marked in amount, has changed from a cellular form to connective tissue made up of fibers and spindle-shaped cells. In the glomeruli the amount of connective tissue in the

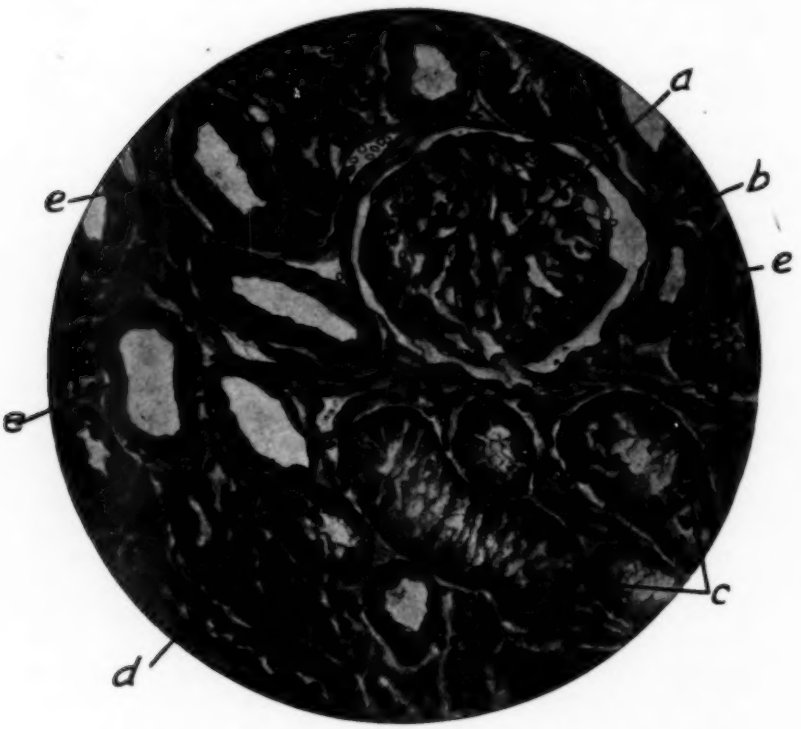

Fig. 2.—Camera lucida drawing, Leitz oc. 2, obj. 6. The figure is from the kidney of the animal of Experiment 8, Table 7, Group 2. The animal has partially recovered from an acute kidney injury from uranium with the development of a chronic nephropathy. The output of urine was normal. The urine contained a trace of albumin and casts. The reserve alkali was 8, phenolsulphonephthalein elimination 38 per cent. There was a slight retention of blood urea and creatinin. At A is shown a glomerulus with the capillaries matted together and an increase in connective tissue nuclei. At B is shown an early thickening of the capsule. At C are shown two of the original convoluted tubules that are undergoing degeneration. At D the intertubular connective tissue is hyperplastic. At E are shown tubules lined by regenerated, flattened, atypical and less specialized epithelium.

form of fibers has increased. Many of the capillary loops have become obliterated, and fibrous adhesions have formed between the capillary tufts and the capsule. The capillaries show a decrease in fibroblasts, but an increase in thickness from the laying down of connective tissue fibers. The periglomerular fibrosis at this stage of the injury consists of newly-formed connective tissue fibers, rich in fibroblasts.

The terminal pathology resulting from the repair of the acute kidney injury leads to the development of a chronic type of nephropathy, which is characterized by a replacement in the damaged tubules of a flattened and less specialized type of epithelium and by a permanent injury to the glomeruli through the formation of connective tissue which is both intracapillary and capsular in location (Fig. 2).

SUMMARY OF THE ANIMALS OF GROUP II, WHICH RECOVERED FROM THE ACUTE RENAL INJURY WITH THE DEVELOPMENT OF A CHRONIC NEPHROPATHY

1. The ability of the members of this group to recover has depended upon the age of the animal. The older animals become anuric, develop an atypical type of air hunger breathing and die either in coma or in a period of convulsions which may precede the coma. The younger animals of the group, after a period of severe renal injury, have recovered with the development of a chronic nephropathy.

2. The earliest evidence of a beginning recovery has consisted in the reappearance of phenolsulphonephthalein in the urine. As the percentage output of the dye increases, there occurs an increase in the alkali reserve of the blood, and an associated increase in the tension of carbon dioxid in alveolar air. There is an attempt to reestablish the normal acid-base equilibrium of the blood. In nine of the animals the acid-base equilibrium has been restored to the normal.

3. Associated with the above evidence of an improvement in renal function there occurs an increase in the output of both acetone and diacetic acid in the urine.

4. After the kidney has established a certain degree of functional improvement there occurs, first, a decrease in the retention of blood urea, and second, a decrease in the retention of creatinin.

5. At the termination of all of the experiments, including those animals in which the alkali reserve of the blood was restored to the normal, there existed marked evidence of renal injury, as was shown by the low output of phenolsulphonephthalein and some degree of retention of urea and creatinin.

6. The kidneys of those animals which have recovered to the point of establishing a chronic nephropathy, have shown histologically a regeneration of tubular epithelium, which is atypical and the establish-

ment of chronic changes in the glomeruli which result in an obliteration and thickening of the capillary loops, a thickening of the capsule and the formation of adhesions between the capsule and the capillary tufts.

GROUP III. NATURALLY NEPHROPATHIC ANIMALS IN WHICH AN ACUTE RENAL INJURY HAS BEEN SUPERIMPOSED ON THE CHRONIC PATHOLOGY BY URANIUM OR MERCURIC CHLORID

Forty-five animals have been studied in this group of experiments. The course of four of the experiments which have been selected as representative of the group is detailed in Tables 8, 9, 10 and 11. Following a period of study, two days of which is recorded in the tables, the animals were rendered acutely nephropathic by either uranium or mercuric chlorid. By such a technic an acute renal injury has been superimposed on the chronic injury. The course of the nephropathy is detailed in the tables until the termination of the experiment.

A study of these tables shows that the urine of all the animals contained a trace of albumin and casts. The animals were freely diuretic. The daily output of urine varied from 471 to 1,141 c.c. The reserve alkali has varied between 8.0 to 8.1. The tension of alveolar air carbon dioxid has shown a correlation with this finding, and has varied between 38 to 42 mm. With the present interest which is being shown both in the laboratory and the clinic in studies of the acid-base equilibrium of the blood in the various nephropathies, the observation just made is of importance. Animals with certain types of chronic kidney injury may have a normal reserve alkali of the blood. In all of the animals the output of phenolsulphonephthalein has been low. The elimination has varied between a maximum output of 53 per cent. and a minimum output of 33 per cent. The findings in this group of naturally nephropathic animals in so far as the reserve alkali of the blood and the elimination of phenolsulphonephthalein is concerned show a close resemblance to the observations made in the animals of Group II that recovered from an acute kidney injury with the development of a chronic nephropathy. The tables of experiments show furthermore that in these animals with a chronic naturally acquired nephropathy, even with a phenolsulphonephthalein elimination as low as 48 per cent., there may be no marked retention of either blood urea or creatinin. The blood urea in these animals has varied between 19 to 22 mg., and the creatinin between 2.0 to 2.31 mg. From these observations it is clear that in the dog a chronic nephropathy may exist with no evidence of impaired renal function other than appearance in the urine of a trace of albumin with casts and a persistently low output of phenolsulphonephthalein.

Six animals with such functional findings have been killed and the pathology of the kidney studied. The histological changes in the

kidneys of these animals have been similar to those previously described in another paper.[7] The chronic pathology is very largely confined to the glomeruli. The glomeruli show both intracapillary and capsular changes. The tufts of capillaries are infiltrated with fibroblasts. The

TABLE 8.—GROUP III, EXPERIMENT 16. NATURALLY NEPHROPATHIC ANIMAL. ACUTE KIDNEY INJURY FROM URANIUM

Animal: Age, 8 to 10 years.

Day of Exp.	Water in 24 Hrs., C.c.	Nephrotoxic Agent	Urine in 24 Hrs., C.c.	Albumin and Casts	Acetone, Mg. per 100 C.c.	Diacetic Acid, Mg. per 100 C.c.	Blood Urea, Mg. per 100 C.c	Blood Creatinin, Mg. per 100 C.c.	Phthalein 2 Hrs., per Cent.	R. p. H	Carbon Dioxid Tension, Mm.
1	500	0	505	Trace Occasional cast	0	0	21	2.31	49	8.0	40
2	500	0	742	Trace Occasional cast	0	0	18	2.10	51	8.0	40
3	500	4 mg. uranium per kg.	618	3.2 gm. Casts very numerous	0	0	18	2.14	Trace	7.9	30
4	500	0	42 later anuric	4.0 gm. Casts very numerous	Present	Present	48	4.00	0	7.85	25
5	500	0	0	Not made	Not made	Not made	72	4.75	0	7.7	14

TABLE 9.—GROUP III, EXPERIMENT 4. NATURALLY NEPHROPATHIC ANIMAL. ACUTE KIDNEY INJURY FROM URANIUM

Animal: Age, 7 years, 2 months.

Day of Exp.	Water in 24 Hrs., C.c.	Nephrotoxic Agent	Urine in 24 Hrs., C.c.	Albumin and Casts	Acetone, Mg. per 100 C.c.	Diacetic Acid, Mg. per 100 C.c.	Blood Urea, Mg. per 100 C.c	Blood Creatinin, Mg. per 100 C.c	Phthalein 2 Hrs., per Cent.	R. p. H.	Carbon Dioxid Tension, Mm.
1	500	0	471	Trace Occasional cast	0	0	19	2.0	45	8.1	42
2	500	0	1141	Trace Occasional cast	0	0	17	2.5	41	8.05	42
3	500	4 mg. uranium per kg.	760	1.3 gm. Numerous casts	5.173	1.672	28	2.59	Trace	7.9	30
4	500	0	419	4.0 gm. Numerous casts	0	0	40	2.70	0	7.8	22
5	500	0	158	3.6 gm. Numerous casts	0	0	78	3.74	0	7.75	15
6	500	0	0 Anuric	Not made	Not made	Not made	121	5.32	0	7.7	10

capillary walls are thickened from a laying down of intercapillary connective tissue. Many of the capillary loops are obliterated. The capsular epithelium is frequently hyperplastic and the capsule is rich in fibroblasts and thickened by fibrous tissue. A periglomerular fibrosis has frequently been observed. The tubular epithelium is histologically well preserved. Many of the convoluted tubules appear normal but in

such epithelium fat can be constantly demonstrated by Scharlach R. In other convoluted tubules the epithelium is of the flattened, atypical, regenerated type, which has been previously discussed. The cells of the ascending limb of Henle's loop contain fat. In these naturally

TABLE 10.—GROUP III, EXPERIMENT 22. NATURALLY NEPHROPATHIC ANIMAL. ACUTE KIDNEY INJURY FROM MERCURIC CHLORID

Animal: Age, 3 years, 7 months.

Day of Exp.	Water in 24 Hrs., C.c.	Nephrotoxic Agent	Urine in 24 Hrs., C.c.	Albumin and Casts	Acetone, Mg. per 100 C.c.	Diacetic Acid, Mg. per 100 C.c.	Blood Urea, Mg. per 100 C.c.	Blood Creatinin, Mg. per 100 C.c.	Phthalein 2 Hrs., per Cent.	R. p. H.	Carbon Dioxid Tension, Mm.
1	500	0	1141	Trace Occasional cast	0	0	21	2.0	48	8.0	38
2	500	0	740	0.5 gm. Occasional cast	0	0	30	2.0	61	8.0	40
3	500	15 mg. mercuric chlorid per kg.	150	1.25 gm. Occasional cast	0	0	34	2.10	12	7.8	28
4	500	0	81	1.5 gm. Numerous casts	0	0	42	2.75	0	7.7	10
5	500 vomited	0	0 Anuric	Not made	Not made	Not made	74	4.68	0	7.65	10 (—)

TABLE 11.—GROUP III, EXPERIMENT 11. NATURALLY NEPHROPATHIC ANIMAL. ACUTE KIDNEY INJURY FROM MERCURIC CHLORID

Animal: Age, 4 years, 2 months.

Day of Exp.	Water in 24 Hrs., C.c.	Nephrotoxic Agent	Urine in 24 Hrs., C.c.	Albumin and Casts	Acetone, Mg. per 100 C.c.	Diacetic Acid, Mg. per 100 C.c.	Blood Urea, Mg. per 100 C.c.	Blood Creatinin, Mg. per 100 C.c.	Phthalein 2 Hrs., per Cent.	R. p. H.	Carbon Dioxid Tension, Mm.
1	500	0	712	Trace No casts	0	0	22	2.15	53	8.0	40
2	500	0	405	0	0	0	18	2.0	51	8.0	40
3	500	0	1142	0	0	0	24	2.10	33	8.0	40
4	500	15 mg. mercuric chlorid per kg.	714	Trace Occasional case	0	0	23	2.0	12	8.0	40
5	500	0	105	0.25 gm. Occasional cast	0	0	41	3.15	0	7.85	26
6	500	0	0	Not made	Not made	Not made	74	4.25	0	7.8	25
7	500 vomited	0	0	Not made	Not made	Not made	112	4.30	0	7.8	23
8	500 vomited	0	0	Not made	Not made	Not made	184	5.06	0	7.75	10

nephropathic animals with the pathologic changes in the kidney very largely confined to the glomeruli, the functional response may be normal with the exception of the presence of albumin in the urine and the low output of phenolsulphonephthalein (Fig. 3).

The further study of the group of naturally nephropathic animals has consisted in producing an acute nephropathy with either uranium in one dose given subcutaneously of 4 mg. per kilogram, or by employing mercuric chlorid in one dose of 15 mg. per kilogram. The mercury was given to the animals by a stomach tube after they had been par-

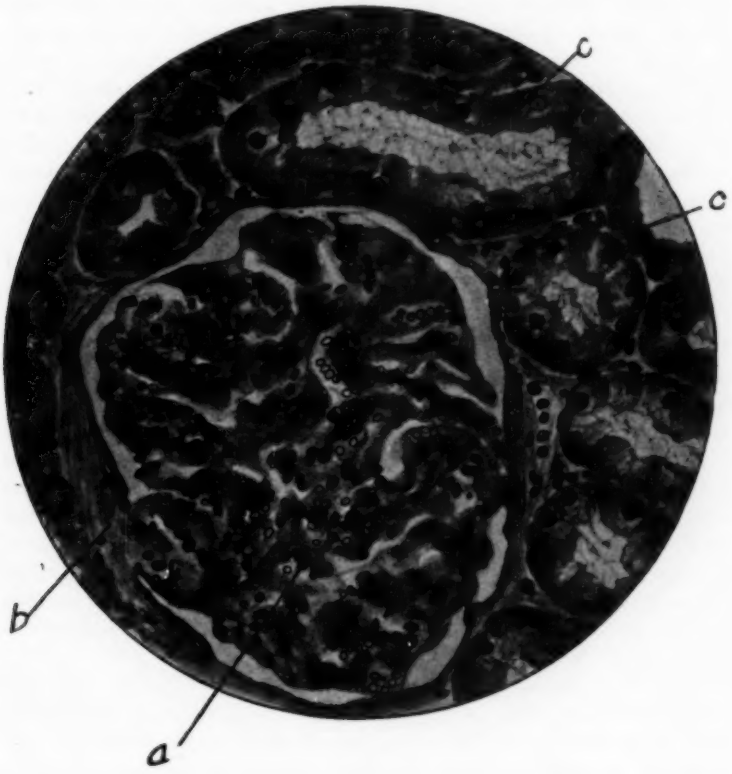

Fig. 3.—Camera lucida drawing, Leitz oc. 2, obj. 6. The figure is from the kidney of one of six naturally nephropathic animals of Group III that were killed after a period of study and before an acute kidney injury was produced by either uranium or mercuric chlorid. The animal had an advanced chronic glomerulonephropathy with but little injury to the tubular epithelium. The daily output of urine was normal. The urine contained a trace of albumin and rarely a hyaline cast. The reserve alkali was 8. The elimination of phenolsulphonephthalein was 49 per cent. There was no retention of either blood urea or creatinin. At A is seen a very large glomerulus with capillary loops thickened and matted together. At B is shown the thickening of the capsule and an adhesion between the capsule and the glomerular tuft. At C are shown convoluted tubules well preserved histologically. There is but slight granulation of the cytoplasm. The nuclei stain well.

tially narcotized by morphin. In a recent paper [19] it has been shown that mercuric chlorid in such a dose produces an acute epithelial injury to the kidney and that this injury is associated with a rapid reduction in the reserve alkali of the blood.

A study of the four tables of experiments representing this group shows that on the day following the use of either one of these nephrotoxic agents, there occurs a sharp decrease in the output of urine, and that the amount of albumin in the urine and the number of casts shows an increase. Associated with this change in the urine there occurs a rapid depletion in the alkali reserve of the blood and a marked reduction in the elimination of phenolsulphonephthalein. In the animal of Experiment 16 that received uranium, the output of urine was reduced on the first day from 742 to 618 c.c., and the albumin increased from a mere trace to 3.2 gm. per liter. The reserve alkali was reduced from 8.0 to 7.9 and the elimination of phenolsulphonephthalein from 51 per cent. to a trace which could not be determined. In the animal of Experiment 22, that received mercuric chlorid, the output of urine was reduced during the first day from 740 to 150 c.c., and the amount of albumin in the urine increased from 0.25 gm. to 1.25 gm. per liter. In this same period the reserve alkali of the blood was reduced from 8.0 to 7.8 and the elimination of phenolsulphonephthalein from 61 to 12 per cent. By the second day of the acute renal injury there had occurred a retention of both urea and creatinin. In the animal of Experiment 22 that received mercuric chlorid the same type of response occurred, though to a less marked extent. During the remaining one to four days of these experiments there occurs a continuation of these functional changes and a further disturbance in the acid-base equilibrium of the blood. By the second or the third day all of the animals had become anuric and remained anuric until the termination of the experiments.

The experiments were terminated on the third to the fifth day following the use of the acutely acting nephrotoxic agent. During this period the reserve alkali of the blood was progressively depleted until such low readings as 7.7 to 7.65 were obtained in the various experiments. There was an associated decrease in the tension of alveolar air carbon dioxid. The elimination of phenolsulphonephthalein was early in the experiments reduced to zero and remained negative on the succeeding days. The retention of blood urea and creatinin showed a progressive increase.

At the termination of the experiments kidney tissue was obtained for histologic study. The vascular pathology of the kidney in these

19. MacNider, William deB.: A Study of Acute Mercuric Chlorid Intoxications in the Dog, with Special Reference to the Kidney Injury, J. Exper. Med. **27**:519, 1918.

animals with a naturally acquired chronic nephropathy that have received either uranium or mercuric chlorid, has shown no evidence of an acute injury other than the engorgement of the glomerular vessels. The vascular pathology is essentially that of a chronic glomerulonephropathy. The acute changes in the kidney that have been superimposed on the chronic pathology consist in an acute injury to the

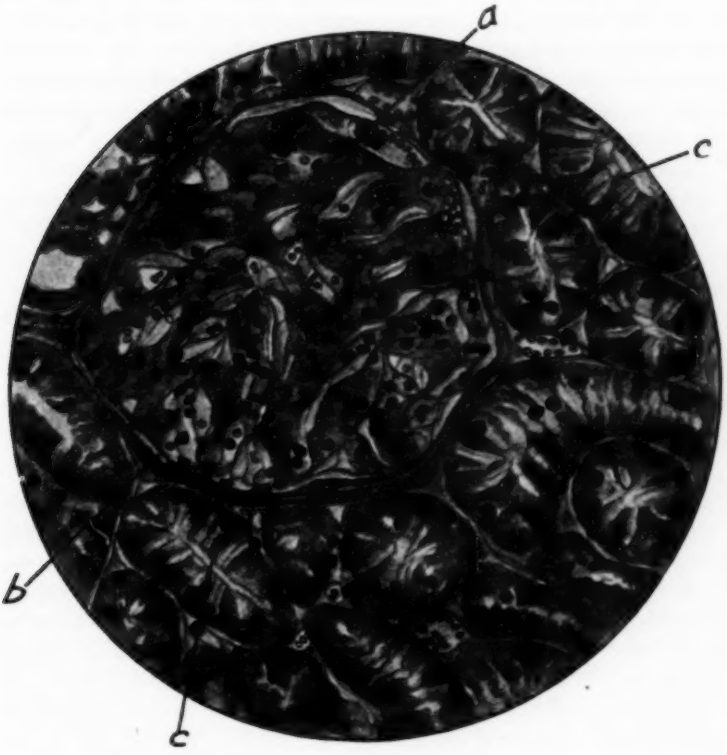

Fig. 4.—Camera lucida drawing, Leitz oc. 2, obj. 6. The figure is from the kidney of Experiment 11, Table 11, Group III. The animals had a naturally acquired chronic glomerulonephropathy. An acute tubular nephropathy was superimposed on the chronic pathology by mercuric chlorid. The animal became anuric on the third day following the use of mercuric chlorid. The reserve alkali of the blood was reduced to 7.7. The phenolsulphonephthalein elimination in a two hour period was negative. There was a retention of blood urea of 184 mg. and of creatinin of 5.05 mg. per 100 c.c. of blood. At A is shown a large glomerulus. The capillary walls are thickened and matted together. The glomerulus contains numerous connective tissue nuclei. At B is shown an early thickening of the capsule of the glomerulus. At C are shown convoluted tubules the epithelium of which is severely swollen and vacuolated and beginning to undergo necrosis.

tubular epithelium, and especially of the proximal convoluted tubules and the ascending limb of Henle's loop. These changes consist in an acute swelling, with granulation and vacuolation of the cells which is rapidly followed by necrosis. The convoluted tubule epithelium has shown a moderate amount of fat, while the epithelium of the ascending limb of Henle's loop has contained fat in a much larger amount. The acute injury produced in the naturally nephropathic kidney by the use of uranium or mercuric chlorid is confined to the tubular epithelium. The functional response of the kidney associated with this injury is characterized by a rapid reduction in the output of urine and an increase in the amount of albumin and number of casts. At the same time that this change occurs, the reserve alkali of the blood shows a marked depletion and the elimination of phenolsulphonephthalein is rapidly reduced to zero. Following this functional disturbance a rapid retention of both blood urea and creatinin takes place which increases until the termination of the experiment. In such naturally nephropathic animals with an acute tubular injury there has been no histological evidence in the kidney, and no functional response to indicate a reestablishment of kidney function (Fig. 4).

SUMMARY FOR THE NATURALLY NEPHROPATHIC ANIMALS OF GROUP III IN WHICH AN ACUTE KIDNEY INJURY WAS SUPERIMPOSED ON THE CHRONIC INJURY BY URANIUM OR MERCURIC CHLORID

1. The type of chronic naturally acquired nephropathy which has been observed in the dog is characterized anatomically by an injury to the glomerulus which is out of proportion to the severity of the changes in the renal epithelium. A similar observation has been made by Stengel, Austin and Jonas [8] for certain nephropathies in human material.

2. The functional response of the kidney in this type of injury is characterized by a low phenolsulphonephthalein output and by the presence in the urine of a small amount of albumin. Casts have been constantly present.

3. In such nephropathies with the injury very largely confined to the glomeruli and with an elimination of phenolsulphonephthalein which has varied between 53 to 38 per cent. there has not occurred a retention of either blood urea or creatinin. From this observation it would appear that for this type of chronic vascular injury the phenolsulphonephthalein elimination test is of more value than are the retention tests of blood urea and creatinin. It would furthermore appear that the injury to the kidney as indicated by the elimination of phenol-

sulphonephthalein must be of a severe type before kidney function becomes sufficiently impaired to indicate the injury by a retention of these substances.

4. Naturally nephropathic animals with but slight histologic evidence of injury to the tubular epithelium, but with a marked glomerulonephropathy, may be able to maintain a normal acid-base equilibrium of the blood as is indicated by the reserve alkali of the blood and the tension of carbon dioxid in alveolar air. Such an observation is, however, no index of the stability of the acid-base equilibrium. When such animals are subjected to the action of agents as uranium or mercuric chlorid that are known to disturb the acid-base equilibrium in normal animals, in these naturally nephropathic animals the depletion of the blood of its alkali reserve occurs more suddenly and to a greater extent than is the case in normal animals. The acid-base equilibrium in a naturally nephropathic animal may be maintained at the point of normality, but when the mechanism which effects such a maintenance is subjected to the strain of maintaining it against an accumulation of acid ions, its lack of stability is shown by a rapid reduction in the alkali reserve with the development of an acid intoxication.

5. Following the use of either uranium or mercuric chlorid in these naturally nephropathic animals there occurs a rapid reduction in the alkali reserve of the blood and a marked reduction in the ability of the kidney to eliminate phenolsulphonephthalein. Within twenty-four hours the elimination of the dye has been reduced to a trace, or it has failed to appear in the urine. Associated with these changes the output of urine is sharply reduced and the amount of albumin in the urine is increased. Later during the course of the acute kidney injury, from one to three days after the initial reduction in the output of phenolsulphonephthalein, there develops a rapid retention of both blood urea and creatinin. These animals become rapidly anuric. During the development of the anuria, the reserve alkali of the blood continues to be depleted, no phenolsulphonephthalein appears in the urine, and the retention of blood urea and creatinin show a progressive increase.

6. At the termination of such experiments the histologic study of the kidneys has shown the essential pathology which develops in the naturally nephropathic animals from the use of uranium or mercuric chlorid to consist in an acute tubular injury without any acute injury having been superimposed on the chronic glomerular pathology. From this observation the deduction appears permissible that the functional capacity of the naturally nephropathic kidney, as indicated by the ability of such a kidney to form urine and to eliminate phenolsulphonephthalein, urea and creatinin is more dependent on the histologic preservation of the renal epithelium than it is on the preservation of the normal structure of the glomeruli.

GENERAL DISCUSSION

The foregoing study has been concerned with two main points: The value of certain tests for renal function in definite types of acute and chronic renal disease, and of the influence of the disturbance in the acid-base equilibrium of the blood which occurs in these nephropathies on the pathology of the kidney and on its functional response.

In our earlier studies on renal function, tests other than those employed in the present investigation were made use of. These tests included a study of the elimination of sodium chlorid, lactose and potassium iodid as first used by Schlayer and Takayasu [20] and later employed by Rowntree, Fitz and Geraghty.[21] In addition to these tests, studies of renal function were conducted by employing Ambard's coefficient for urea elimination as modified by McLean [22] and extensively used in the studies of Mosenthal and Sclater.[23] In our later studies, including those found in this paper, these tests have not been employed. In our experience, the elimination of sodium chlorid, lactose and potassium iodid have been so variable, even in nephropathies in which one fairly definite type of injury was found to exist, that their value has become questionable. The use of Ambard's coefficient has been discontinued as a functional test, for it gives less accurate information of the ability of the kidney to secrete a substance than does the phenolsulphonephthalein test. Furthermore, as has been recently pointed out by Addis and Watanabe,[24] the rate of urea excretion in man varies in a manner which cannot be explained by the concentration of urea in the blood and urine. For these reasons, we have confined our observations to the use of the phenolsulphonephthalein test as an index of the immediate functional capacity of the kidney and have used estimations of blood urea and creatinin as indices of retention.

These tests have been employed in animals with a naturally acquired or experimentally produced nephropathy. The experiments were terminated at different periods during the renal injury and observa-

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20. Schlayer and Takayasu: Untersuchung über die Funktion kranker Nieren, Deutsch. Arch. f. klin. Med. **93**:17, 1909.

21. Rowntree, L. G., Fitz, R., and Geraghty, J. T.: The Effects of Experimental Chronic Passive Congestion on Renal Function, Arch. Int. Med. **11**: 121, 1913.

22. McLean, Franklin C.: The Mechanism of Urea Retention in Nephritis, Studies from the Rockefeller Institute for Medical Research, **28**:549, 1918. Nephritis from the Standpoint of Urea Excretion, Studies from the Rockefeller Institute for Medical Research, **28**:569, 1918.

23. Mosenthal, Herman O., and Sclater, Lewis D.: A Comparative Study of Tests for Renal Function, J. A. M. A. **67**:933 (Sept. 23) 1916.

24. Addis, Thomas, and Watanabe, C. K.: Rate of Urea Excretion. A Criticism of Ambard's and Weill's Laws of Urea Excretion, J. Biol. Chem. **24**:203, 1916.

tions made on the functional capacity of the kidney in connection with a definite pathologic state as found in the kidney. In an animal with a pure tubular injury as is produced by uranium in the early days of the intoxication, the first functional disturbance on the part of the kidney consists in a reduction in the elimination of phenolsulphonephthalein, the appearance of albumin in the urine and associated with these functional changes there is a reduction in the reserve alkali of the blood. There is a definite relationship between the injury to the tubular epithelium, the reduction in the elimination of phenolsulphonephthalein and the appearance of albumin and casts in the urine. At this stage of such an experiment there is no glomerular injury. At this early period of such experiments, it is difficult to state just what the relationship is from the standpoint of causation, between the reduction in the reserve alkali of the blood and the establishment of the injury to the tubular epithelium. By the methods which have been employed in this study, our observation has been that the reduction in the reserve alkali of the blood and the development of the tubular injury either occur at the same time or the depletion of the alkali reserve of the blood occurs prior to the development of the tubular injury, as is shown by the elimination of phenolsulphonephthalein, and the appearance of albumin and casts in the urine. This early change in the acid-base equilibrium of the blood should not be looked on as a disturbance due to retention from an injury to the kidney. The naturally nephropathic animals have shown that the acid-base equilibrium may be normal and yet there may be a decrease in the elimination of phenolsulphonephthalein which is much lower than is the reduction in the elimination of the dye when the early disturbance in the acid-base equilibrium of the blood occurs.

An investigation [25] concerning the way in which uranium induces the kidney injury appeared some years ago. In this study it was shown that the toxic effect of uranium for the tubular epithelium of the kidney could be prevented by the use of intravenous injections of sodium carbonate prior to the use of uranium. If the disturbance in the acid-base equilibrium of the blood was prevented by the use of such an alkaline solution, uranium lost its selective and toxic effect for the renal epithelium. It was furthermore shown that such an alkaline solution had no detoxicating effect on a solution of uranium, for the uranium could be dissolved in an alkaline solution, injected subcutaneously in the usual dose and produce the acute tubular nephropathy. The way in which uranium induces a depletion of the alkali reserve

25. MacNider, William deB.: The Inhibition of the Toxicity of Uranium Nitrate by Sodium Carbonate; and the Protection of the Kidney Acutely Nephropathic from Uranium from the Toxic Action of an Anesthetic by Sodium Carbonate, J. Exper. Med. **23**:171, 1916.

of the blood which in turn is associated with the development of an injury to the tubular epithelium is now under investigation. Very recently [26] the observation has been made that in intoxications by both uranium and mercuric chlorid which induce a kidney injury that is associated with a depletion in the alkali reserve of the blood, the liver shows evidence of injury prior to the development of the kidney injury. The injury to the liver is associated with the earliest disturbance in the acid-base equilibrium of the blood. When we consider the importance of the liver in connection with oxidations, the above observation may be of importance in explaining the disturbance in the acid-base equilibrium of the blood which in turn is associated with the development of the epithelial degeneration in the kidney.

In these acutely nephropathic animals with primarily a tubular injury the elimination of phenolsulphonephthalein is rapidly reduced, and after the functional capacity of the kidney as is indicated by the output of this dye has been seriously disturbed, there occurs a retention, first of urea which is followed within twenty-four to forty-eight hours by a retention of creatinin. From this observation we infer that the phenolsulponephthalein test is a more sensitive index of renal function than are the retention tests of blood urea and creatinin. The further inference appears allowable, that all of these substances, phenolsulphonephthalein, urea and creatinin, are eliminated by the renal epithelium. This conclusion is furthermore strengthened by the study of the elimination of these substances in animals that have recovered from an acute tubular nephropathy (Group II), and by the group of naturally nephropathic animals (Group III) which have been rendered acutely nephropathic. In the animals that recover from uranium the process of recovery is characterized anatomically by a regeneration of tubular epithelium and by obliterative and sclerotic changes in the glomeruli. Associated with these anatomic findings the functional recovery of the kidney is shown first by the reappearance and an increase in the elimination of phenolsulphonephthalein, and later, when further regeneration of renal epithelium has occurred; but when more extensive destructive changes have taken place in the glomeruli, there occurs a decrease in the retention of both urea and creatinin.

The group of naturally nephropathic animals which were rendered acutely nephropathic by uranium or mercuric chlorid has shown a similar type of functional response. These animals had a definite chronic glomerulonephropathy which was out of proportion to the degree of tubular injury. In such animals there was a moderate reduction in the elimination of phenolsulphonephthalein and blood urea and

26. MacNider, William deB.: On the Occurrence of Degenerative Changes in the Liver in Animals Intoxicated by Mercuric Chlorid and Uranium Nitrate, Proc. Soc. Exper. Biol. & Med. **16**:82, 1919.

creatinin were either normal or showed a slight retention. When, however, an acute tubular injury was superimposed on the chronic vascular pathology by the use of uranium or mercuric chlorid, there occurred a sharp reduction in the elimination of phenolsulphonephthalein which in eight animals became negative within twenty-four hours, and also a rapid retention of both urea and creatinin.

A study of the disturbance in the acid-base equilibrium of the blood in the later stages of the acute nephropathy from uranium, shows that as the severity of the tubular injury increases, there is a progressive decrease in the alkali reserve of the blood and an associated reduction in the tension of carbon dioxid in alveolar air. This disturbance may go to the point of placing the animal in a state of air hunger and coma. During this depletion in the alkali reserve of the blood, there appears in the urine both acetone and diacetic acid. After a certain degree of functional disturbance has been produced in the kidney, the elimination of these ketone bodies is decreased and associated with this change in their quantitative output, there occurs a further reduction in the alkali reserve of the blood. From this observation it is inferred that a part of the depletion of the alkali reserve of the blood which occurs late in a tubular injury of the kidney is due to a retention of acid bodies which is dependent upon the inability of the severely damaged tubular epithelium to eliminate such substances. This inference is strengthened by the further observation that when animals recover from the acute tubular injury by the regeneration of tubular epithelium, as is shown by an increase in the elimination of phenolsulphonephthalein and by a decrease in the retention of urea and creatinin, there occurs an increase in the output in the urine of both acetone and diacetic acid. Associated with the increased elimination of these bodies, there occurs an increase in the alkali reserve of the blood and an increase in the tension of alveolar air carbon dioxid.

With the improvement in renal function of animals recovering from an acute uranium nephropathy there is a continued increase in the alkali reserve of the blood which may reach the point of normality. In such animals, even though sufficient regeneration of tubular epithelium has occurred to maintain a normal acid-base equilibrium, the existence of the chronic nephropathy is indicated by the low output of phenolsulphonephthalein and by the retention of urea and creatinin. A similar observation has been made for the naturally nephropathic animals. Such animals with a marked glomerulonephropathy and relatively well preserved tubular epithelium may show a normal alkali reserve of the blood and yet the elimination of phenolsulphonephthalein may be low and there may or may not be a retention of urea and creatinin. The existence of a normal alkali reserve of the blood is,

however, no index of the stability of the acid-base equilibrium. When such animals are given either uranium or mercuric chlorid, there occurs a very rapid depletion of the alkali reserve with an associated injury to the tubular epithelium, a very rapid reduction in the elimination of phenolsulphonephthalein, a retention of blood urea and creatinin and a decrease in urine formation which soon leads to the establishment of an anuria.

CONCLUSIONS

1. The acute tubular nephropathy which is produced by uranium is associated with the ability of the metal to effect a disturbance in the acid-base equilibrium of the blood. The tubular injury is more marked in an old animal than in a young animal. In an old animal the depletion of the alkali reserve of the blood occurs more rapidly and is more pronounced than is the case in a young animal.

2. This injury to the kidney is indicated functionally, first by a reduction in the elimination of phenolsulphonephthalein and later by a retention of blood urea and creatinin.

3. Asssociated with the establishment of such an injury both albumin and casts appear in the urine. The amount of albumin in the urine is no index of the severity of the pathology in the kidney or the degree of functional disturbance. The quantitative output of albumin may show a progressive decrease, while at the same time the elimination of phenolsulphonephthalein is rapidly decreasing, and both urea and creatinin are showing a retention.

4. A certain number of the animals rendered acutely nephropathic by uranium have recovered from the acute injury with the development of a chronic nephropathy. Those animals which effected a recovery were young animals. The old animals were unable to establish and further those changes of repair which lead to recovery with a chronic nephropathy. The changes in the kidney during the period of recovery have consisted in the regeneration of tubular epithelium of a flattened and less specialized type and of the production of chronic obliterative and sclerotic changes in the glomeruli. With the establishment of this type of chronic injury there occurs an increase in the alkali reserve of the blood which may reach the point of normality. There is an increase in the elimination of phenolsulphonephthalein and a decrease in the retention of blood urea and creatinin.

5. From these anatomic and functional observations the inference is drawn that the tubular epithelium is of more importance in maintaining a normal acid-base equilibrium of the blood, and is more concerned with the elimination of phenolsulphonephthalein, urea and creatinin than is the vascular mechanism of the kidney.

6. Similar observations have been made in a group of naturally nephropathic animals in which the chronic injury to the kidney has consisted in a glomerulonephropathy with histologically well preserved tubular epithelium. In such animals the reserve alkali of the blood may be normal, the elimination of phenolsulphonephthalein may be only slightly reduced, and there may be no retention of blood urea and creatinin. When, however, an acute tubular injury is superimposed on this chronic injury by uranium or mercuric chlorid there occurs an early edema and necrosis of the tubular epithelium without the development of any acute injury to the glomeruli. Such an injury has been associated with a rapid depletion of the alkali reserve of the blood and an associated decrease in the tension of carbon dioxid in alveolar air. The acute injury is expressed functionally by a rapid reduction in the elimination of phenolsulphonephthalein and by a retention of blood urea and creatinin. The animals become acutely anuric and die in convulsions or in a coma which may not be preceded by convulsions.

7. The present investigation, which has been concerned with certain functional and anatomical studies in different types of nephropathic processes, tends to minimize the importance of the glomerulus as a functional unit and to emphasize the relative importance of the tubular epithelium. The investigation furthermore points out the influence of a disturbance in the acid-base equilibrium of the blood on the histology and functional capacity of the renal epithelium.

HIGH PROTEIN DIETS AND ARTERIOSCLEROSIS IN RABBITS

A PRELIMINARY REPORT *

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In studies dealing with the effect of high protein diets on the kidneys of rabbits,[1] it was noted that the animals which ate such diets for a number of months, showed at necropsy widespread arteriosclerosis of the aorta. It was further noted that the extent of the vascular disease was roughly proportional to the duration of the high protein diet.

Thus, the notes from a group of rabbits fed a mixture whose protein content was made high by means of casein [2] gives the following information in regard to the condition of the aorta:

Rabbit 56: Weight, 2,950 gm.; casein diet three weeks. Aorta normal.

Rabbit 54: Weight, 2,270 gm.; casein diet, two and one-half months. Aorta normal.

Rabbit 61: Weight, 2,500 gm.; casein diet, eleven months. The arch and thoracic portion of the aorta show many raised plaques and streaks.

Rabbit 57: Weight, 1,815 gm.; casein diet one year. The root, the ascending limb of the arch and to a lesser extent the thoracic aorta, show many irregular raised yellow plaques and streaks.

Rabbit 58: Weight, 2,725 gm.; casein diet one year. The root, the ascending limb and the arch of the aorta show many discrete and confluent raised yellow patches and streaks.

In another group of rabbits fed a diet consisting of a mixture of dried powdered beef and bread flour in the proportion of one to two, the notes dealing with the condition of the aorta read as follows:

Rabbit A-1: Weight, 2,522 gm.; meat diet four weeks. Two very small raised yellow patches in the thoracic aorta.

Rabbit B-1: Weight, 2,000 gm.; meat diet six weeks. Aorta shows one small patch of arteriosclerosis about 2 mm. in diameter.

Rabbit A-2: Weight, 1,240 gm.; meat diet three months. Beneath each cusp of the aortic valves is a raised pale yellow plaque. The orifices of some of the vessels leaving the aorta are surrounded by raised irregular yellow rings. A number of raised patches are seen in the arch of the aorta.

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1. Newburgh, L. H.: The Production of Bright's Disease by Feeding High Protein Diets, Arch. Int. Med. **24**:359 (Oct.) 1919.

2. The mixture consisted of milk, water, scraped carrot, sodium bicarbonate and casein.

Rabbit B-2: Weight, 2,090 gm.; meat diet four and one-half months. Small raised patches occur at the opening of every vertebral artery and at the openings of the subclavians. A number of raised yellow patches are seen in the arch of the aorta.

Rabbit A-4: Weight, 2,380 gm.; meat diet seven months. The thoracic aorta shows very many pale yellow raised streaks and plaques. These areas are so numerous in the arch that they form a continuous layer.

Rabbit B-3: Weight, 2,870 gm.; meat diet seven months. The thoracic aorta from the bases of the valves to the diaphragm shows many large raised pale yellow patches. A few similar patches are seen in the abdominal aorta.

The two obvious sources of error in assuming that the vascular lesion observed bears a causal relationship to the diet are: first, that arteriosclerosis may be a lesion of very frequent occurrence in laboratory rabbits; and second, that arteriosclerosis may be common in old rabbits, and that the experimental rabbits in which the arteriosclerosis was found were old and would, therefore, be expected to show a high incidence of this vascular disease.

The frequency of spontaneous arteriosclerosis in laboratory rabbits has been investigated by several authors. Hedinger and Loeb [3] state that "spontaneous arterial disease has not been observed in rabbits so far as has been shown by the literature. We ourselves have never found such lesions in about one hundred rabbits that were investigated with that in view." Miles,[4] on the other hand, found aortic lesions in seventeen of forty-nine (34.6 per cent.) supposedly normal rabbits. She adds that rabbits obtained from a dealer raising them for the market in large numbers and in close quarters showed a higher percentage of lesions than those obtained from other sources. Bailey [5] studied the effect of diphtheria toxin on the arteries of rabbits. Thirty-five animals were used. Three of these were controls and showed normal vessels. The thirty-two others were treated with the intention of producing vascular lesions. Of these the vessels were normal in twenty-seven. Hence, in 84 per cent. of rabbits treated by Bailey with the intention of producing arteriosclerosis, no vascular lesions were found.

The experience of other workers who have investigated the frequency of arteriosclerosis in supposedly normal rabbits may be summarized by the statement that vascular lesions may be expected in occasional rabbits assumed to be normal, but that spontaneous arteriosclerosis is not an important source of error when the majority of a group of rabbits shows vascular lesions.

In order to control our observations still further, we have examined the aortas of fifty-nine rabbits, twenty of which had been used in the

3. Hedinger and Loeb: Arch. f. exper Pathol. **56**:314.
4. Miles: Spontaneous Arterial Degeneration in Rabbits, J. A. M. A. **49**: 1173, 1907.
5. Bailey: J. Exper. M. **25**:109, 1917.

laboratory course in pharmacology and the remainder of which had been used in the laboratory of internal medicine. The aortas of all these rabbits, with two exceptions, were normal. The first exception was formed by a rabbit whose aorta presented a single raised patch, 1 mm. in diameter. This animal had been in the laboratory seven and one half months. It had had "snuffles" during all these months, had been immunized against the typhoid bacillus seven months previously, and at necropsy was found to have pneumonia. The second abnormal aorta showed a small, slightly raised, sclerotic patch near an aortic cusp, and several very small raised yellow patches in the arch. The outcome of the investigation of this large number of control animals fully justifies the statement that arteriosclerosis of the aorta is a very uncommon lesion in untreated rabbits.

The second possible source of error, namely, that arteriosclerosis may be common in senile rabbits, will not account for the vascular lesions found in the group of animals which ate the meat mixture, since these rabbits were not senile. Their high protein diet was begun when they were about 6 months old. Consequently, the oldest of them could not have been more than 13 or 14 months old when killed.

The proportionality existing between the length of the high protein feeding and the amount of vascular injury, serves to emphasize the view that the high protein diet was either directly or indirectly the cause of the arteriosclerosis.

A description [6] of the microscopic appearance of the lesion kindly furnished me by Dr. C. V. Weller reads as follows: "Rabbit 58.—Aorta. Hemalum and eosin and Van Gieson's stains. The intima is thickened irregularly through the presence of elevated plaques, many of which are three or four times as thick as the normal aortic wall. These elevated patches in most cases involve only the intima. In a few instances, the innermost lamellae of the media are included. The elevated patches show a proliferation of the intima, increase in the elastic fibers and large areas of secondary fatty degeneration and liquefaction necrosis, accompanied by marked formation of cholesterin. The picture is that of an advanced intimal atherosclerosis in varying stages up to and including secondary atheromatous change with cholesterin formation. The greater part of the media and adventitia are negative."

It is evident from this description that we are dealing with true arteriosclerosis as it occurs in the human being.

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6. This description was published as part of a footnote in the paper entitled "The Production of Bright's Disease by Feeding High Protein Diets, loc. cit.

THE ALKALI RESERVE IN PELLAGRA

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In the urinary findings of thirty-one cases of pellagra studied at the U. S. Pellagra Hospital, Spartanburg, S. C., in the spring and summer of 1917, fourteen patients showed high ammonia and low urea ratios. Of the fourteen high ammonia ratios, the ammonia was relatively increased in eight, while in the remaining six the urinary output of ammonia was much increased over that of four normal persons serving as controls. In some cases the ammonia nitrogen ran as high as 20 per cent. of the total nitrogen. In a number of cases subsequent to 1917, a high ammonia ratio was found at the time the patient entered the hospital.[1]

Though it was well recognized that a high ammonia ratio, whether the ammonia is relatively or absolutely increased, is susceptible of other explanations than a depletion of the normal alkali reserve of the body, or a state of acidosis, the urinary findings spoken of suggested that in some cases, at least, there might be a greater or less depletion of the alkali reserve.

In regard to acidosis in pellagra, Fairbanks[2] believes that pellagra is caused by an acidosis resulting principally from phosphoric acid and purins in the blood. Carmichael,[3] on the contrary, considers that the disease is due to an increase in the alkalinity of the blood caused by the excessive ingestion of fruits and vegetables. Jobling and Maxwell,[4] using the Van Slyke method (they do not state how the blood was collected) found the average carbon dioxid volume per cent. to be 61.63 in twenty acute cases and 57.27 in sixteen chronic cases, and concluded that the alkali reserve of the blood in pellagra does not vary from normal in either the acute or the chronic cases and that, therefore, there is no acidosis or alkalosis in pellagra. Very recently there came to our notice a British report on pellagra[5] wherein is reported some evidence of acid intoxication in pellagra as deduced from the facts that there was found a slight increase in the ammonia and in titratable acid of the urine in twenty early cases of pellagra, using phenol-

1. The results of the urine work will be published later.
2. Texas State M. J. **21**:170, 1916.
3. Quoted by Jobling and Maxwell: J. A. M. A. **69**:2026 (Dec. 15) 1917.
4. Loc. cit.
5. Report of a Committee of Inquiry Regarding the Prevalence of Pellagra Among the Turkish Prisoners of War, p. 36, Sec. B, 1918.

phthalein as indicator, and in tests in eight early cases of pellagra and four well marked cases a slight decrease in the fixed alkali of the blood as determined by titrating with dimethyl-amino-azobenzene as indicator.

The study of the alkali reserve here was started late in the summer of 1917, subsequent to the findings of a tendency to a heightened ammonia ratio and subsequent to the crest of the pellagrous wave for that season. During these last two seasons pellagra was far less abounding and the cases in the hospital were far less severe than in previous years.

In the study herein described two methods of procedure were employed: First, the determination of the venous carbon dioxid tension by the Plesch [6] method as modified by Higgins [7] and described in detail by Marriott; [8] and second, the carbon dioxid bound by the blood plasma by the Van Slyke and Cullen [9] method using the Van Slyke [10] carbon dioxid apparatus.

For the sake of brevity and quick elucidation of the alveolar air method, we may quote Van Slyke and Cullen [11] who cover the subject so well.

"1. Arterial carbon dioxid tension (Haldane method). The alveolar air as shown by A. and M. Krogh is in equilibrium in respect to its carbon dioxid content with the arterial blood. Consequently, in accordance with the law of gas solubility the concentration of carbon dioxid in the alveolar air is directly proportional to that of free carbonic acid in the blood. Consequently, the carbon dioxid concentration of the alveolar air is through the intermediary parallelism of the blood carbonic acid, kept proportional to arterial sodium bicarbonate All three concentrations go up and down together, the blood bicarbonate fixing level of the carbonic acid, and the latter that of the alveolar carbon dioxid. Under pathologic conditions, or under the influence of drugs, of decreased atmospheric oxygen tension, or of anxiety or excitement, the sensitiveness of the respiratory control may vary so that the alveolar carbon dioxid is not under all conditions even an approximate measure of the bicarbonate reserve. All sources of error together, however, even in pathologic conditions, are in most cases encountered within such limits that the clinical utility of carbon dioxid determinations as measure of the alkali reserve of the blood is thoroughly established, although the fact that so many factors beside the alkaline reserve of the blood can affect the alveolar carbon dioxid tension certainly make the latter far from an ideal measure of the former.

"2. Venous carbon dioxid (Plesch method). The Plesch method differs from the Haldane in that the air analyzed, instead of being taken at the end of a single quick expiration, is breathed in and out of a rubber bag by the subject for from thirty to forty seconds. Consequently, the carbon dioxid tension approaches more nearly that of the venous than of the arterial blood, the Plesch results being, as a rule, from 4 to 6 mm. higher in carbon dioxid ten-

6. Ztschr. Exper. Pathol. u. Therap. **6**:380, 1909.
7. Carnegie Institute of Washington, Publ. 203, p. 168, 1915.
8. J. A. M. A. **66**:1594 (May 20) 1916.
9. J. Biol. Chem. **30**:289, 1917.
10. Ibidem, 347.
11. Loc. cit., pages 299, 300. See this article for references to alveolar air determination under normal and pathological conditions.

sion than the Haldane results. Since the venous carbon dioxid tension runs fairly parallel with the arterial, however, the Plesch results may be taken as indirect measures of the arterial bicarbonate and, so to say, one degree less direct than the Haldane. An advantage of the Plesch technic is that it requires less cooperation on the part of the subject than the Haldane procedure."

The Plesch method was used by us. In the details on the method of collecting the samples of air and the technic of the method of analysis, etc., the directions given by Marriott [8] were followed closely. This method determines the venous carbon dioxid tension, that is, the concentration of carbon dioxid in the alveolar air in equilibrium with the carbon dioxid content of the venous blood. In acidosis the concentration of the carbon dioxid of the alveolar air is diminished.

In normal adults at rest, the carbon dioxid tension in the alveolar air, determined by the Plesch method, varies from 40 to 45 mm. Tensions between 30 and 35 mm. are indicative of a mild degree of acidosis. When the tension is as low as 20 mm. the individual may be considered to be in imminent danger. We regard values of 35 mm. and over as normal. The Plesch method, as pointed out by Van Slyke and Cullen, may be taken as an indirect measure of the alkali reserve. The Van Slyke method of determining the carbon dioxid bound by the blood plasma is a direct determination of the alkali reserve of the body, that is, the excess of base which is left after all the nonvolatile acids have been neutralized and is available for the immediate neutralization of further acids.

ALVEOLAR AIR METHOD

The venous carbon dioxid tension was determined (1) on patients who had been in the hospital some time and had shown marked improvement; (2) on patients who had been in the hospital a longer or shorter time but with a slower change for the better, and (3) on patients recently admitted and still in a marked stage of pellagra. The condition of the patients from a pellagra standpoint, irrespective of their condition at the time of entering the hospital (which condition, of course, was noticeably pellagrous for them to obtain admission), was noted at the time the tests were made. The only treatment, barring marked complication, was nutritional. The diet of the patients varied. At the time of testing some had been on a very generous hospital diet with meat, milk, eggs, fruits, etc.; others had been on a similar hospital diet, but with less milk, meat, etc., while still others, who were tested on or very shortly after admission, had been on a rather slim diet, as far as could be ascertained, with little meat, milk or eggs. As the diet seemed to play little part in the degree of acidosis, the diet records, where known, are not herein listed.

TABLE 1.—Venous Carbon Dioxid Tension in Pellagra

Case No.	Sex	Age	Carbon Dioxid Tension	Attack	Ammonia Nitrogen, Total Nitrogen, Per Cent.	Status of Pellagra at Time of Testing
1918						
325	M.	48	40.0	1	4.99	Cured
337	M.	46	37.5	1	7.00	Cured
341	F.	18	40.0	1	5.14	Cured
353	F.	30	35.0	0	8.22	Considerable improvement; now a moderate case
357	F.	35	42.5	1	4.73	Much improved. Now a mild case
358	F.	26	37.5	1	6.66	Practically well. Skin lesions practically cured. Now a mild case
369	M.	37	37.5	1	4.86	Improving; still some skin lesions; now a moderate case
370	M.	57	35.0	2	8.72	Markedly pellagrous
371	F.	65	37.5	2	4.91	Moderately pellagrous
372	M.	44	35.0	2	3.38	Very markedly pellagrous
375	M.	47	42.5	4	6.92	Very markedly pellagrous
376	M.	34	32.5	2	2.07	Weak and moderately pellagrous; residual skin lesions
378	F.	29	40.0	3	10.32	Moderately pellagrous; slight skin lesions
1919						
539	M.	65	32.5	1	3.2	Convalescing slowly; considerable skin lesions; now a moderate case
557	M.	60	35.0	2	....	Practically cured
563	M.	45	32.5	1	....	Practically cured. Given cascara several hours previous to test
570	F.	23	32.5	5	....	Practically cured. Nursing an infant
571	M.	59	25.0	2	4.3	Pellagra cured. Asthmatic
572	F.	14	32.5	1	....	Practically cured
573	M.	50	32.5	7	....	Practically cured
574	M.	60	35.0	1	5.5	Cured
576	F.	43	35.0	2	4.0	Almost well; now a very mild case
581	F.	29	32.5	2	10.8	Almost well; now a very mild case
583	F.	27	32.5	2	3.6	Cured. Nursing an infant
585	F.	39	32.5	6	4.2	Improving rapidly; now a mild case
586	F.	56	32.5	2	10.0	Improving, desquamating; now a mild case
587	M.	47	27.5	2	3.0	Markedly pellagrous, complicated with edema
1919*						
1	M.	54	32.5	2	3.7	Mild case
3	F.	50	32.5	1	8.7	Marked case
5	M.	30	32.5	1	4.9	Mild case. Asthmatic
7	M.	68	42.5	1	2.2	Very mild case
10	F.	24	35.0	1	8.8	Moderate case, desquamating. Occasionally nurses 14 months old infant
11	F.	36	32.5	2	3.3	Mild case. Nursing an infant
12	F.	48	32.5	1	6.0	Moderate case, desquamating
13	M.	44	40.0	..	4.7	Sent in as pellagrous. Diagnosis not confirmed
15	F.	39	42.5	4	3.1	Mild case, desquamating. Nursing an infant
16	M.	28	30.0	1	4.0	Marked case, desquamating
17	F.	18	25.0	1	5.8	Severe; complicated with thyroprivia
19	M.	58	42.5	2	3.2	Moderate case, desquamating
20	F.	28	42.5	2	15.8	Mild case, desquamating
21	F.	15	37.5	4	4.6	Moderate case, slight erythema
22	F.	38	40.0	9	3.0	Mild case, desquamating
23	F.	21	37.5	1	6.6	Mild case, desquamting
24	F.	41	37.5	3	2.12	Mild case, desquamating
25	F.	20	32.5	1	12.8	Mild case

* A new series of case numbers was started July 1, 1919.

The average venous carbon dioxid tension of the forty-four pellagra patients tested by the alveolar air method was 35.23 mm. Leaving out four complicated cases, two patients with asthma, one patient with beriberi symptoms, and one patient with thyroprivia, complications which might affect the alveolar air determinations, the average of the forty uncomplicated cases was 36.00 mm. Of these forty patients, twenty-four, or 60 per cent., had a venous carbon dioxid tension of 35 mm. or over with an average of 38.44 mm. In sixteen cases,

or 40 per cent., the carbon dioxid lay between 30 and 35 mm., suggestive of a mild degree of acidosis. Five of these sixteen patients, however, when further tested by the Van Slyke method for carbon dioxid bound by the blood plasma gave carbon dioxid values of practically 60 volume per cent. and over, so there was no lack of alkali reserve. Ten of the forty patients tested by the alveolar air method were cured of pellagra at the time the test was made, that is, the erythema and roughness of the skin had disappeared, the patients had gained in weight and had a general feeling of well being and were to be discharged shortly. The average carbon dioxid tension of these ten patients was 35 mm. The average of the other thirty cases, with slight or marked pellagra, was 36.33 mm. This figure is probably slightly lower than it should be, since a few of the patients in 1919 were tested in the afternoon, when the results would undoubtedly be slightly lower than if taken in the morning. All the determinations, too, were made in the hot summer when some salt loss might be expected by reason of sweating.

TABLE 2.—Venous Carbon Dioxid Tension in Pellagra Patients Arranged in Four Groups

Very Marked Pellagra, Mm.	Moderate Pellagra, Mm.	Mild Pellagra, Mm.	Cured of Pellagra, Mm.	Average of Twenty-One Controls, Mm.
35.0	35.0	42.5	40.0	
42.5	37.5	37.5	37.5	
42.5	37.5	35.0	40.0	
32.5	32.5	32.5	35.0	
30.0	40.0	32.5	32.5	
	32.5	32.5	32.5	
	35.0	32.5	32.5	
	32.5	42.5	32.5	
	42.5	32.5	35.0	
	37.5	42.5	32.5	
		42.5		
		40.0		
		37.5		
		37.5		
		32.5		
Average 35.0	36.25	36.88	35.0	39.76
Per cent. 35 mm. or over 60.0	70.0	60.0	50.0	90.48

The relation of acidosis to pellagra, as tested by the alveolar air method, is further brought out in Table 2 in which the patients are grouped, according to their condition when the tests were made, into those with marked pellagra, those with moderate pellagra, those with mild pellagra, and those who were considered cured of pellagra.

Despite the fact that whatever error there might be in our technic would tend to make our results low, it may be seen (1) that the majority of the hospital patients tested by the alveolar method of testing acidosis was normal; (2) that in those cases where the carbon dioxid tension was somewhat subnormal, the subnormality as shown in

Table 2, had nothing to do with the severity of the pellagra symptoms as judged primarily by the extent of the erythema and dermatitis and secondarily by the state of malnutrition and general weakness, and (3) that in general the patients gave slightly lower venous carbon dioxid tension than did the controls [12] who were regarded as normal.

The twenty-one nonpellagrins as controls, normal as far as known, gave venous carbon dioxid tensions from 32.5 (two cases) to 45 mm., with an average of 39.76 mm.

CARBON DIOXID BOUND BY THE BLOOD PLASMA

The Van Slyke-Cullen method of estimating the carbon dioxid of the blood plasma was used. The blood was drawn from the arm vein directly into a centrifuge tube containing potassium oxalate (about 0.5 per cent. of the weight of the blood drawn) and paraffin oil. The blood was stirred by means of the inlet tube and centrifuged immediately, and the carbon dioxid of the plasma was determined in duplicate samples of 1 c.c. The results are given in Table 3.

As may be seen from Table 3, the carbon dioxid bound by the blood plasma of the thirty patients (exclusive of Case 13) varied from 45.3 to 69.5 volume per cent., with a general average of 60.05. This average is almost identical with that of thirty-six pellagra patients tested by Jobling and Maxwell [3] at the Davidson County Pellagra Hospital, Tenn. They found that the average carbon dioxid volume per cent. was 61.63 for twenty acute cases, and 57.27 for sixteen chronic cases, which gives a general average of 59.69 for all their patients.[13]

According to Van Slyke and Cullen,[9] with the method employed by us (venous blood drawn under oil without stagnation of blood or loss of carbon dioxid) the minimal normal figure is 60 c.c. of carbon dioxid per 100 c.c. of plasma. Of the thirty pellagra patients tested by this method at the U. S. pellagra Hospital, twenty, or 66.67 per cent., were practically 60 volume per cent. or over, and ten, or 33.33 per cent., were below 60. The average of the ten cases below 60 was 52.4 per cent. carbon dioxid, with only two cases appreciably below 50 per cent. Of the ten cases below 60 per cent. of carbon dioxid,

12. The controls were chemists, physicians, nurses, clerks, and attendants who had been connected with the hospital for a shorter or a longer time over the period the tests on the patients were made. All the controls were active, nonpellagrous people, with a feeling of well being and in normal health, as far as known. In their general appearance, state of nutrition, and joy of living the controls were much superior to the patients.

13. We hesitate to apply the term "chronic" to our cases since, though in many cases there had been recurrences in the spring and summer, there were, in the intervening colder months, periods of apparent well being and a toning up of the general condition and lack of erythema of the skin, in short, periods when pellagra was not manifest. Hence, the recurrences are spoken of as such and such an attack.

three (142a, 401a and 332a) were practically negative as far as external manifestations of pellagra were concerned though the patients were still rather weak; three (481, 567 and 20) were mild cases; one (502) was a moderate case, while only two cases (485 and 488) were severe.

TABLE 3.—Carbon Dioxid Bound by Blood Plasma

Case	Sex	Age	Percentage of Carbon Dioxid Bound by Plasma	No. of Attacks	Ammonia Nitrogen / Total Nitrogen, per Cent.	Status of Pellagra at Time of Testing
142a	M.	43	45.3; b89.9	2	....	Feeling dull and out of condition at time of first test but convalescing with skin neg.
401a	M.	37	47.5; b62.2	2	....	Receiving dilute hydrochloric acid per os at first test, weak, improving, slight skin lesions, loose bowels
471	F.	25	61.5	1	....	Improving, residual skin lesions
479	F.	40	66.4	1	....	Improving, desquamating and atrophic skin
480	M.	63	59.9	2	5.51	Improving, desquamating and residual skin lesions
481	M.	49	50.4	3	....	Improving, residual skin lesions; now a mild case
332a	F.	29	57; b56.9	2	5.01	Weak and anemic, slight skin lesions
484	M.	52	67.9	1	....	Severe, skin lesions marked
485	M.	32	50.9; b59.1	1	6.57	Severe, skin lesions marked
488	F.	30	50.4; b59	1	....	Severe, skin lesions marked
496	M.	33	59.4	1	5.83	Mild case
497	M.	36	60.0	2	20.6	Skin lesions marked
500	F.	37	59.9	8	5.65	Skin lesions marked
501	M.	31	64.7	1	16.12	Skin lesions marked
502	M.	58	56.5	1	....	Skin lesions moderate
567	M.	53	53.0	1	....	Almost well, now a mild case
585	F.	39	60.0	6	4.2	Markedly pellagra, desquamating
587	M.	47	58.8	2	3.0	Marked pellagra with edema
1919						
1	M.	54	66.6	2	3.7	Mild case
3	F.	50	67.7	1	8.7	Marked case
5	M.	30	61.7	1	4.9	Mild case
7	M.	68	67.4	1	3.2	Very mild case
10	F.	24	66.8	1	8.8	Occasionally nurses 14 mos. old infant. Moderate case; desquamating
11	F.	36	59.5	2	3.3	Mild case. Nursing an infant
12	F.	48	69.5	1	6.0	Moderate case, desquamating
13	M.	44	66.0	...	....	Sent to hospital as pellagrous (diagnosis not confirmed)
15	F.	39	60.1	3	3.1	Mild case, desquamating. Nursing an infant
16	M.	28	61.0	1	4.0	Marked case
19	M.	58	68.4	2	3.2	Mild case
20	F.	28	54.6	2	15.8	Mild case
22	F.	38	68.6	9	3.7	Mild case

a. Second test of alkali reserve after another month in the hospital, with marked improvement in general condition and with the pellagra symptoms negative or practically so.

It would seem, then, that in the cases of pellagra studied the carbon dioxid bound by the blood plasma was for the most part normal, that in a minority of cases the carbon dioxid bound by the plasma was slightly subnormal, but that this subnormality was not relatable to the severity of the pellagra symptoms.

Of the seventy uncomplicated cases of pellagra tested by the two methods, venous carbon dioxid tension and carbon dioxid bound by the blood plasma, fourteen cases were duplicates by the two methods. Of the fifty-six separate cases tested for alkali reserve by the two methods, none showed a marked depletion of the alkali reserve, about one-third showed a slightly subnormal level, while the greater number of cases were within normal limits.

THE RELATION OF THE URINARY AMMONIA RATIO TO THE CARBON DIOXID TENSION AND THE CARBON DIOXID BOUND BY THE BLOOD PLASMA

In Tables 1 and 3, the urinary ammonia ratio is given for a number of cases at or near the time the tests specifically designated in the two tables were made. A number of the patients had previously a very high ammonia ratio, but only the ratio at the time of the carbon dioxid analyses were made is herein considered. The analysis of normal urines here showed that the ammonia nitrogen did not go above 7 per cent. of the total nitrogen. Accordingly, ratios of ammonia nitrogen to total nitrogen above 7 per cent. were regarded as high, 7 per cent. or below 7 per cent. as normal. Of the thirty-eight cases included in Table 1, analyzed for carbon dioxid tension and urinary ammonia and total nitrogen, only four patients showed concomitantly a high ammonia ratio and a low carbon dioxid tension; five patients had a high ammonia ratio and a normal carbon dioxid tension; twelve patients had a low ammonia ratio with a low carbon dioxid tension; while seventeen patients had a low ammonia ratio with a normal carbon dioxid tension. Similarly, in Table 3 dealing with carbon dioxid bound by the plasma, the ammonia ratio was over 7 per cent. of the total nitrogen in five cases. Only one of these five patients (Case 20) showed a low percentage of carbon dioxid bound by the plasma, namely, 54.6 per cent. The other four patients with high ammonia ratio (Cases 497, 501, 3 and 10) showed a normal percentage of carbon dioxid bound by the plasma. On the other hand, three cases (332a, 485 and 587) showed a lowered alkali reserve with a normal urinary ammonia ratio. Thirteen of the cases analyzed for both factors, or approximately 62 per cent., were normal both as to ammonia and as to the carbon dioxid bound by the plasma.

It would seem, then, from a consideration of the relation of the ammonia ratio to the carbon dioxid tension of the alveolar air and the carbon dioxid bound by the plasma, that, barring other diagnostic factors, the urinary ammonia ratio is not a measure of acidosis or lack of alkali reserve.

GENERAL CONCLUSIONS

Of the fifty-six patients tested for alkali reserve by the alveolar air method and by the determination of the carbon dioxid bound by the blood plasma, none showed a marked depletion of the alkali reserve, about one-third showed a slightly subnormal level, while the greater number were within normal limits. There would seem to be little uncompensated acidosis in pellagra.

RECOVERY FROM TUBERCULOUS MENINGITIS AFTER TREATMENT WITH INTRASPINAL INJECTIONS OF ANTIMENINGOCOCCIC SERUM *

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The treatment of tuberculous meningitis has always been more or less haphazard, largely symptomatic, and entered upon by the clinician with very little enthusiasm and with even less hope of success because of the very general opinion of the uniform fatality of the disease. It has been possible, however, to collect from the literature about forty cases of unquestionable tuberculous meningitis terminating in recovery, and we wish to report four cases of the disease which have terminated in recovery.

It will not be within the scope of this paper to review the literature of recoveries from tuberculous meningitis, as this has been most admirably done by Alfred E. Martin [1] in 1909. His critical survey of the reports on such cases up to that date show that of thirteen cases with remission, varying from four months to five years, and terminating in death, only eight were undoubted cases of tuberculous meningitis; and of the cases ending in permanent recovery, only twenty-five were definitely proven to be cases of tuberculous meningitis. Some writers, as Babinski and Strumpel, strongly maintain that in a large proportion of the reported cases of recovery the diagnosis is incorrect, being merely cases of serous meningitis, and it is proven in that article that such is the case. During recent years, the greater exactness of diagnosis of this disease, due to the increased use of the spinal puncture as a diagnostic measure combined with refinements in examination of the fluid, has resulted in a paucity of reports of cured cases.

A careful search through the medical literature from 1910 to date has resulted in finding eleven cases ending in recovery, which have been definitely proven to be tuberculous, the criteria being the clinical course, the cell count and differential of the spinal fluid, the finding of tubercle bacilli, and the demonstration of the disease in an inoculated guinea-pig; the last two being the only real proof. Those who have reported these cases are Castaigne and Gourand,[2] Barbier and

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1. Martin, A. E.: Occurrence of Remissions and Recovery in Tuberculous Meningitis, Brain **32**:209, 1909.

2. Castaigne and Gouraud: J. Méd. Français, May 15, 1911.

Gougelet,[3] Elsner[4] having seen a case at Treupel's clinic; Reichmann and Rauch[5] had two cases, Pitfield,[6] Schaeffer,[7] Archangelsky,[8] Fonzo,[9] Tilli[10] and Dunn.[11] All of these cases were clinically and bacteriologically cases of tuberculous meningitis, and as all of the reporters used different methods of treatment, it was interesting to note the almost universal agreement that there was an improvement following removal of spinal fluid. There are also on record seven cases in which the diagnosis has not been confirmed by the recovery of the Koch bacillus from the spinal fluid, the diagnosis having rested either on the presence of tuberculosis elsewhere, the clinical course, or the cutaneous tuberculin reaction. Those who report these cases are I. Celeste,[12] Bernard and Debré,[13] Di Rinzi,[14] and Browning[15] four cases.

To the above we add the two undoubted and two doubtful cases reported in this paper. A summing up of these statistics shows that there are thirty-eight cases ending in recovery in which the diagnosis is certain, and fifteen in which it is doubtful. With these cases we will have to consider those cases ending in recovery which have never been contributed to the literature, but even with them it is not a very hopeful total in view of the apparent frequency of the disease.

STATISTICS

Statistical reports from two children's hospitals show a 100 per cent. mortality, the disease in children, as in extra miningeal tubercu-

3. Barbier, H., and Gougelet: Episodes Tuberculous meningitis curables chez les enfants, Bull. et mém. Soc. méd d. hôp. de Par. **31**:440, 1911.

4. Elsner: Tuberculous Meningitis in Monographic Medicine **6**:225.

5. Reichmann and Rauch: Ueber die Prognose und Therapie der Meningitis Zwei geheilte Faelle von Meningitis Tuberculosis, München. med. Wchnschr. **9**:1374, 1430 (July 1) 1913.

6. Pitfield, R. L.: Recovery from Tuberculous Meningitis, with Report of Cases, Am. J. M. Sc. **146**:73, 1913.

7. Schaeffer, S.: Tuberculous Meningitis; a Case with Recovery, New York M. J., Jan. 11, 1913.

8. Archangelsky, W. G.: Zur Frage über die Moeglichkeit einer Heilung der Tuberculosen Meningitis, Jahrb. f. Kinderh. **74**:155, 1911.

9. Fonzo, F.: Sulla Guaribilita della meningitie tubercolare, Pediatria **23**:334, 1915.

10. Tilli, P.: Autoserotherapy of Tuberculous Meningitis, Policlinico, Rome **23**: 1916.

11. Dunn, C. H.: Tuberculous Meningitis, Arch. Pediat. **27**:685, 1910.

12. Celeste, I.: Sulla curabilita della Meningitie tubercolare, Gazz. d. osp., Milano **31**:1137, 1910.

13. Bernard, L., and Debré, R.: Bull. et mém. Soc. méd. d. hôp. de Par. **30**:587, 1910.

14. Di Rinzi, E.: Di un cas de meningitie tubercolare sequito da guarigione, Boll. d. Clin., Milano **27**:367, 1910.

15. Browning, C. C.: Four Cases of Tuberculous Meningitis with Arrest, Med. Rec. **86**:325, 1914.

losis, being more virulent than in adults. Holt [16] mentions such statistics, as does A. E. Meyers [17] in a series of 105 cases, the patients being under 10 years of age. Statistics [1] collected from a large London General Hospital and from one in Vienna show nearly the same results, except for several indefinite cases ending in recovery. A careful analysis of the cases on the pediatric and medical services at St. Luke's Hospital, from 1898 to date, show a uniform fatal result, except for the few cases we report. There have been ninety-three cases in patients under 15 years of age, with no recoveries, while among patients over 15 years there were forty-five deaths and four recoveries. This makes a total of 142 cases during the past 20 years, with a mortality of 99.2 per cent. In the face of these discouraging reports, it is not surprising that writers on this disease consider recovery an impossibility. They do not seem to appreciate that there is an opportunity, however small, for remission or cure by prompt and unremitting treatment, and they are usually content to make a diagnosis and treat their hopeless patient symptomatically.

TREATMENT OF DISEASE

There have been many suggestions for therapy in this disease, but this very multiplicity reveals their inefficacy. Among these are a legion of drugs which have been found of value in other forms of tuberculosis, such as forced feeding, application of leeches to the mastoid, tuberculin and hexamethylenamin for its supposed bactericidal effect in the spinal fluid. In the prewassermann years these patients were frequently given large doses of potassium iodid (600 grains per day), and mercury by mouth or by inunction. This is still persisted in by some clinicians, but the refinements in the diagnosis of neurosyphilis enable us to differentiate between these two conditions. Operation in the form of decompression and opening into the cisterna magna has been advocated by C. W. Stiles [18] and H. E. Alexander,[19] being compared by the latter to a laparotomy for the relief of peritoneal tuberculosis, while its relief of pressure recommends it to the former.

Since the introduction of spinal puncture by Quincke, in 1891, this procedure has come to be looked on more and more as a therapeutic as well as a diagnostic aid, so that it is now largely employed for the relief, even though temporary, of all forms of increased intra-

16. Holt, L. E.: Tuberculous Meningitis, Am. J. Dis. Child. **1**:26, 1911.

17. Meyers, A. E.: Tuberculous Meningitis. A Study in Children; 105 Cases, Am. J. Dis. Child. **13**:427 (May) 1915.

18. Stiles, C. W.: Tuberculous Meningitis, Proc. Sixth Internat. Cong. Tuberc., 1908.

19. Alexander, H. E.: Tuberculous Meningitis, The Postgraduate, N. Y. **27**: 769, 1912.

cranial pressure whether from uremia, "wet brain," encephalitis, brain tumor or meningitis. In the large series of children noted above, Meyers [11] reports that those treated by repeated spinal puncture showed a longer duration of the disease, and to this therapeutic method both Riebold and Rumpel ascribe the cure of their cases. Pitfield's [6] patient showed great immediate improvement following the withdrawal of 80 c.c. of spinal fluid with subsequent complete recovery. Oppenheim, in his "System," [20] in 1910, says, "there is in my opinion no longer room for doubt as to the possibility of recovery from tuberculouus meningitis," and recommends repeated spinal punctures as the most promising method of cure. In the cases which have come under our observation, this procedure has always been followed by relief of the symptoms even though for a short time only, so it is our belief that spinal puncture, frequently repeated, should be considered a real asset in the treatment of tuberculous meningitis.

The injection of 30 c.c. of a 1 per cent. solution of iodoform in almond oil into the subdural space every six hours, has been recommended by one author, but does not seem to warrant commendation. P. Tilli,[10] basing his theory on the subcutaneous injection of chest fluid in tuberculous pleurisy, has used autoserotherapy by injecting subcutaneously from 1 to 3 c.c. of spinal fluid every three days. He reported three cases, one of which, a child, 18 months old, recovered. The rationale of this procedure does not seem very clear, and it is difficult to draw conclusions from one case. F. Raymond [21] has originated a novel procedure which was employed on French soldiers during the recent war; i. e., the injection intraspinally of sterilized air. A spinal puncture is performed, 40 c.c. of fluid are removed, and then 20 c.c. of air (previously passed through a red hot platinum needle for sterilization) is injected into the spinal canal. This is repeated on five or six consecutive days. No cures were reported. The improvement which was obtained following the treatment was possibly due to the spinal drainage accompanying it. Other Frenchmen, Bernard and Debré,[13] have recommended the intraspinal injection (after the removal of spinal fluid) of 5 mg. of sulphate of radium, repeated frequently. They report one case ending in recovery, the diagnosis of which, however, must be classed as doubtful.

One of us (A. W. H.), in 1916, was confronted with a question of diagnosis in the first case of our series, and in the interim gave many intraspinal injections of antimeningococcus serum. The very satisfactory termination of this case prompted us to follow a similar procedure in the second case, with again a favorable outcome. In addition

20. Oppenheim, H.: Textbook on Nervous Disease **11**:786.
21. Raymond, F.: Bull. et mém. Soc. méd. d. hôp. de Par. **12**:1058, 1917.

to these two cases we can report two additional cases terminating in recovery. In reviewing the literature we were interested to find a case reported by Schaefer [7] in which the same treatment was employed to good effect.

REPORT OF CASES

CASE 1.—T. McA., male, aged 26, lawyer, was admitted to St. Luke's Hospital March 27, 1916. He had pleurisy nine years before, for the past three months he had anorexia, was drowsy at times, and complained of a general "run down" condition. Ten days before admission headache developed, gradually increasing in severity; he vomited and was drowsy, and three days before admission he vomited again with anorexia.

Physical Examination.—Patient was acutely ill; no rigidity of neck; lungs clear; bilateral Kernig sign; knee jerks sluggish; pulse large and bounding, from 60 to 70; temperature 103 F. Blood count showed slight anemia. Spinal puncture: 40 c.c. slightly turbid fluid removed under increased tension. Cells, 1,400; lymphocytes, 80 per cent.; polymorphonuclears, 20; globulin, plus; no organisms on smear or culture. Wassermann, negative. Twenty c.c. antimeningococcus serum injected. The following day the patient was very drowsy, with severe headache, stiff neck, Kernig very pronounced, knee jerks absent. Fundus examination showed no papillo-edema, no choroid tubercles. The procedure of the previous day was repeated. The third day he was irrational. Temperature, from 101 to 102 F.; pulse, 60. Spinal puncture and intraspinal serum injected. The next three days there was an improvement in symptoms. Temperature, 98.5 F.; pulse, 80. April 3, another intraspinal injection of 18 c.c of serum was given, followed by an exacerbation of all the symptoms, with rise of temperature to 102.2 F. and increased rigidity of the neck and positive Kernig. Joint pains and urticarial rash developed the following day, with vomiting—a true serum sickness, lasting four days with an immediate subsequent abatement of all symptoms of the meningeal irritation. April 20 a mild follicular tonsillitis developed, with temperature between 99 and 104 F. There was then noticed a paresis of the right side of the face and deviation of the tongue to the left, with pains in the left leg and thigh. A spinal puncture taken at that time showed globulin plus with fifty-one cells; lymphocytes, 97 per cent.; polymorphonuclears, 3 per cent. A partial paralysis of the left leg then ensued. The patient never showed difficulty in swallowing or bulbar speech as an evidence of his pontine lesion. Bright and clear mentally. May 9, he was discharged from the hospital with facial weakness entirely recovered from and only a slight weakness in the leg. One month later he was completely recovered and is now strong and well, practicing law in this city.

TABLE 1.—SPINAL FLUID FINDINGS IN CASE 1

Date	Amount, C.c.	Pressure	Character	Cells	Lymphocytes	Polymorphonuclears	Globulin	Serum, C.c.
3/27	45	+++	Turbid	1,400	80	20	+++	20
3/28	8	+++	Turbid	.....	..	..	.....	5
3/29	32	++	Sl. turbid	2,376	75	25	+++	18
4/ 3	40-50	++	Sl. turbid	346	97	3	++	20
4/22	25	+	Clear	51	97	3	+	
4/26	15	+	Clear	28	97	4	+	

At no time were tubercle bacilli found in the spinal fluid and guinea-pig inoculation revealed nothing, so this case must be classed as doubt-

ful. We have considered it as a case of tuberculous meningitis for the following reasons: History of an antecedent pleurisy; the slow onset for three months; its benign beginning, though eventually manifesting the severe clinical course of a meningitis; the development during its course of a fairly well localized crossed paralysis of the Millard-Gubler type; the presence of a moderately low cell count with persistent lymphocytosis and the failure to find any other organisms, though the most careful search was made by Dr. Fameuelener in smear and through culture.

CASE 2.—W. S. P., male, aged 26, publisher. On admission to hospital, Dec. 1, 1916, the patient also gave a history of having had pleurisy when younger. He had been losing weight for several months; presented a lack of initiative, and a general "run down" condition. Ten days previously, he began to have intermittent headaches. Three days later he came to see one of the house staff suffering from severe headache; he had no temperature and was sent home with directions to take a cathartic and acetylsalicylic acid. When admitted to hospital he had severe occipitofrontal headache, weakness, anorexia, irritability and vomiting.

TABLE 2.—SPINAL FLUID FINDINGS IN CASE 2

Date	Amount, C.c.	Pressure	Character	Cells	Lympho-cytes	Poly-morpho-nuclears	Glob-ulin	Serum, C c.
12/2	7	++	Clear	126	100	..	++	
12/2	20	++	Clear	144	100	..	++	20
12/3	15	++	Clear	?	...	..	.....	15
12/5	20	+	Clear	333	95	5	++	20
12/7	20	+	Clear	441	96	4	++	19
12/10	9	.....	.....	?				

Physical Examination.—Young man, acutely ill, restless, irritable, tossing about in bed; no stiffness of neck or Kernig. A tentative diagnosis of typhoid was made because of paucity of signs. The next day his temperature was 102 F.; pulse slow, bounding, of "cerebral" type. Headache continued severe, requiring morphin; nausea, vomiting and somewhat drowsy. Lungs clear, spleen not palpable; no oculomotor disturbance; neck stiff, bilateral Kernig and knee jerks more sluggish, no Babinski. Widal, blood culture and blood Wassermann all negative. Spinal puncture showed clear fluid under increased pressure; 126 cells; lymphocytes, 100 per cent.; globulin, 2 plus; tubercle bacilli found in moderate numbers (Dr. Karl Vogel). Realizing the apparent hopelessness of the case, it was determined to try the therapy which had been so successful in Case 1. That evening, 20 c.c. of meningococcus serum (Flexner) was given intraspinally, and was followed by slight relief of headache and nausea on the next day. December 4 temperature ranged between 100 and 102 F.; pulse, 60; general condition improved. The following day, a 20 c.c. intraspinal serum injection given which was repeated December 7. Both injections were followed by an exacerbation of the signs of meningeal irritation, with subsequent improvement. December 10, following a gradual decline in temperature, an urticarial rash appeared and then severe joint pain and tenderness developed. December 13, the temperature was 99 F.; serum sickness disappeared; general condition showed noticeable lack of cerebral signs with pulse assuming a normal character. December 20 temperature had been normal for a week; no stiffness of neck; Kernig absent; knee jerks more active; patient sitting up in bed; appetite good. Patient left hospital February 10, having gained 15 pounds in weight and feeling well, except for slight weakness of his legs. He served in the army in 1918 and is well today.

This case presents the typical onset and clinical course of a tuberculous meningitis, and the bacilli were found in the spinal fluid in moderate numbers. As in the previous case, a definite improvement followed the intraspinal serum injection and especially after the "serum sickness." This is an undoubted case of recovery from tuberculous meningitis.

CASE 3.—Mrs. J. T., aged 47, practical nurse; was admitted to the service of Dr. Hollis July 2, 1919, with severe headache. Her past history showed: at 18 had "typhoid pneumonia" followed by an osteomyelitis of wrist; a nephropexy at 34, and a panhysterectomy at 38; two attacks of malaria, two and four years previously. For the past two months she has had loss of appetite and weight. Ten days ago she developed an intermittent occipital headache becoming worse at night. Four days before admission she was constipated, nauseated and vomited everything taken since then.

TABLE 3.—SPINAL FLUID FINDINGS IN CASE 3

Date	Amount, C.c.	Pressure	Character	Cells	Lympho-cytes	Poly-morpho-nuclears	Glob-ulin	Serum, C.c.
7/ 2*	15	+	Sl. turbid	56	99	1	+++	
7/ 5	20	++	Sl. turbid	Bloody	...	..	.....	20
7/ 6	20	++	Bl. tinged	70	25	75	++	
7/ 7	30	++	Turbid, yellow	780	26	74	++	25
7/ 8	25	+	Turbid, yellow	1,450	20	80	++	16
7/ 9	35	+	Turbid, yellow	460	32	68	+	25
7/10*	15	++	Turbid, yellow	540	30	70	+	
7/11	20	0	Clear, yellow	621	41	59	+	20
7/15	20	0	Clear, yellow	137	59	41	+	
7/18	15	0	Clear	178	83	17	+	
7/21	15	+	Clear	81	79	21	+	
7/26	15	0	Clear	52	100	—	+	
7/31	15	+	Clear	61	98	2	+	
8/ 4	15	+	Clear	37	100	—	+	
8/ 8	18	+	Clear	27	100	—	+	
8/14	15	+	Clear	22	100	—	+	
8/19	18	+	Clear	65	100	—	+	
8/24	5	Slightly +	Clear	30	100	—	+	
8/30	10	0	Clear	15	100	—	+	
9/ 9	10	0	Clear	12	100	—	+	
9/23	12	0	Clear	26	100	—	+	
10/ 6	10	0	Clear	12	100	—	+	
11/10	7	0	Clear	10	100	—	0	

* Colloidal gold test: 7/2, 0 1 1 1.5 2 1 1 0 0 0; 7/9, 1 1 1 1 1 1 1.5 2.5 2 1.5

Physical Examination.—Well nourished; acutely ill; slight strabismus; reflexes hyperactive; some rigidity of neck and suggestion of beginning Kernig sign; temperature from 100 to 103 F.; pulse, 60. Blood count, urine and Wassermann test were negative. Spinal puncture revealed 15 c.c. fluid under increased pressure, 56 cells; 99 per cent. lymphocytes; globulin, 3 plus; spinal fluid Wassermann negative. The course of the disease from then on was very chronic. The headache and vomiting were the outstanding features of the clinical picture. Intraspinal injection of meningococcic serum was given on five occasions, and repeated spinal punctures were done after that for the relief of the distressing headache. The spinal fluid removed eight days after admission was slightly turbid, yellow, and under pressure; cell count, 540; polymorphonuclears, 70 per cent.; lymphocytes, 30 per cent.; globulin, plus and the colloidal gold chlorid curve of the meningitic type 1 1 1 1 1 1 1.5 2.5 2 1.5. Tubercle bacilli were found in this fluid. At this time the patient was suffering from severe headache and vomiting; was restless; no lethargy. There were no oculomotor disturbances. Stiff neck and Kernig were well marked. The

fundi examined by Dr. A. Wiener showed a blurring of both disks on their nasal margin and a patch of old choroiditis in the left eye; no tubercles. From this time on the temperature gradually approached the normal and symptoms abated until on discharge from the hospital, the spinal fluid showed 12 cells; 100 per cent. lymphocytes with faintly plus globulin. Inoculation of the guinea-pig showed tubercles in the spleen. After leaving the hospital she developed an anxiety state which took a persecutory trend. This cleared up in two weeks and the spinal fluid removed at that time was clear; 10 cells and negative globulin.

The clinical course and laboratory findings in this case leave no doubt in our minds as to the presence of a tuberculous meningitis. The marked cellular response in the spinal fluid, including polynucleosis following the injection of serum, and the rapid fall in cells soon after completion of five intraspinal injections had been given, are noteworthy.

CASE 4.—A. T., aged 28, male, builder. One of us (A. W. H.) saw the patient for several days prior to admission to St. Luke's Hospital, Sept. 18, 1919. History shows frequent tonsillitis; lumbago in 1907; gonorrhea in 1918; syphilis denied. He was discharged from the army April 2, 1919, as a captain after having been in France nine months and having participated in three major engagements. For several months he has been feeling "run down." Two weeks ago awoke with a throbbing occipital headache; three days later he vomited and there was a persistence of the headache, becoming gradually more severe. He was in bed one week before admission to the hospital, complaining of anorexia, restlessness and headache. There was no lethargy and no previous acute infection.

TABLE 4.—SPINAL FLUID FINDINGS IN CASE 4

Date	Amount, C.c.	Pressure	Character	Cells	Lympho-cytes	Poly-morpho-nuclears	Glob-ulin	Serum, C.c.
9/28/19	20	++	Clear, yellow	317	91	9	++	
9/30	20	++	Slightly turbid; yellow	128	86	14	++	20
10/ 2	20	++	Clear	122	97	3	+	20
2d Admission								
11/2	20	+	Clear	316	83	17	+	20
11/5	20	+	Clear	206	88	12	+	10
12/6	15	0	Clear	26	100	..	+	

Physical Examination.—Acutely ill; lungs clear; spleen not palpable; rigidity of neck; reflexes exaggerated; no oculomotor palsies; Kernig sign bilateral; temperature 100 F.; pulse, 60. Spinal punctures made September 19 and 21 both bloody. Blood Wassermann, negative; spinal fluid Wassermann, negative. Blood count, normal. Urine showed marked acetone and diacetic acid. September 28 a spinal puncture revealed 20 c.c. clear yellow fluid under increased pressure. Cells 317: polymorphonuclears, 9; lymphocytes, 91; globulin, 2 plus. Colloidal gold test, 0 5 1 1 1 5 2 1 5 5 0 0 0 0. Ophthalmoscopic examination by Dr. Coleman Cutler revealed a low grade papillo-edema, 1/2 D. Left side more marked than the right. October 12 this had almost cleared up. Two intraspinal injections of antimeningococcic serum were given with real clinical and laboratory improvement. The patient was discharged October 25 free from symptoms and signs.

Two days after discharge, after an exposure, he became nauseated and vomited. Complained of a return of headache and of severe joint pains. Could not take food. Temperature, 100 F.; pulse, from 70 to 80. Stiffness of neck had returned and bilateral Kernig. November 2, spinal fluid clear under pressure. Cells 316: polymorphonuclears, 17; lymphocytes, 83; globulin, 2 plus. Two intraspinal injections of serum were given at three day intervals with an immediate drop in cell count and disappearance of the symptoms. The patient is now well (Feb. 20, 1920).

This case presents some interesting features. The clinical course and the spinal fluid are quite characteristic of tuberculous meningitis — the long, slow onset, the mild manifestations and the low cell count with a lymphocytosis; however, as tubercle bacilli were never found in the spinal fluid or as it failed to produce lesions in the guinea-pig, the diagnosis must rest between an influenzal meningitis, a tubercular meningitis or possibly an epidemic encephalitis, though lacking most of the diagnostic features of the latter disease. The tendency to remission occurring so soon after apparent recovery should be taken as either a true exacerbation of the disease or else in view of the presence of severe joint pains it might be considered an abortive serum sickness lighting up as it did in the other cases the meningeal inflammatory signs. This case, in the absence of finding the tubercle bacilli, must be classed as a doubtful case of tuberculous meningitis.

DISCUSSION

In the above reports of cases there are two, Cases 2 and 3, which are recoveries from unquestionable tuberculous meningitis; the two remaining cases, because of the failure of positive laboratory findings, must be classed as doubtful. Against these must be balanced three cases in which the serum was used during the past four years at St. Luke's Hospital with ultimate death. To this total can be added the recovered case of Schaeffer,[7] in which spinal fluid inoculation was positive, making eight patients in all treated with antimeningococcic serum, five of whom recovered.

It is not our purpose to urge a specific treatment, as the optimum is still far from obtained, but as a method of approach, the intraspinal injection of meningococcic serum, combined with frequent spinal drainage, is a therapy by which the patient seems to have at least a chance. Three of our patients were young men in the middle twenties; two of them had had previous tuberculous lesions from which they recovered, while the other had been through a severe army campaign in France. Their physical condition was splendid, and having a localized meningeal form of tuberculosis were very favorable cases for treatment. However, the course of the disease was far from easy in any of them, and more than one consultant gave discouraging prognoses. The woman was a poor risk from the start, and her recovery seemed largely

dependent on her treatment. Of the patients receiving this method of treatment who died, two were men over 45, in whom the resistance was low, one alcoholic and one arteriosclerotic, and the other was of noticeably poor physique. They were given nine and seven intraspinal injections, respectively, with a resultant reduction in the cell count, symptoms and a prolongation of the course of the disease. The third patient, a young man, aged 27, showed no response after five injections, and rapidly succumbed to his disease.

The theoretical aspect of the therapy seems fairly well founded if we consider the very satisfactory results which have been obtained with autoserum in the treatment of the other chronic meningeal disease, syphilis of the spinal cord. Many who have used the Ogilvie or Swift-Ellis technic are quite satisfied that the benefit obtained therefrom is not from the arsphenamin, but from the irritating effect of the serum introduced. A recent report by Mehrtens and MacArthur [22] on the treatment of neurosyphilis showed that the injection of the patient's own serum resulted in a meningeal irritation, with increase in the cells as high as 2,300. We, therefore, consider that the intraspinal injection of antimeningococcic serum has two distinct actions; first, by adding to the spinal fluid certain antibodies which it is unable to develop itself and, second, by the introduction within the dura of a foreign protein in the form of horse serum. The irritative effect of the latter on the meninges produces a cellular response and a hyperemia about the site of any localized tubercle with beneficial result. It is with this theoretical background and the therapeutic results reported that we urge further investigation along this or similar lines. It has been suggested to us that the patient's own serum be given instead of the antimeningococcic serum; however, the tuberculous antibodies would at best be feeble if they had succumbed to a meningeal disease and one's own blood serum is not in a strict sense a foreign protein.

SUMMARY

1. There are reported in the literature thirty-eight undoubted cases of cured tuberculous meningitis, and fifteen in which the diagnosis is doubtful.

2. Except for these cases, the treatment as shown by hospital statistics has been 100 per cent. ineffectual.

3. Therapy has always been experimental in type in hopes of finding a cure.

22. Mehrtens, H. G., and MacArthur, C. G.: Therapy of Neurosyphilis, etc., Arch. Neurol. & Psychiat. **2**:369 (Oct.) 1919.

4. The authors have employed intraspinal injections of antimeningococcic serum, combined with frequent spinal drainage, and report two cases of tuberculous meningitis and two others possibly tuberculous meningitis showing recovery under this method.

5. Theories as to the results are discussed.

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LETHARGIC ENCEPHALITIS: SYMPTOMATOLOGY AND HISTOPATHOLOGY *

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ST. PAUL MINNEAPOLIS

Our present literature is teeming with symptomatology and case reports of lethargic encephalitis. Since v. Economo,[1] in the spring of 1917, reported his first thirteen cases in detail, similar clinical conditions have been observed on the Eastern Continent as well as in this country. It first manifested itself on our Eastern coast in the fall of 1918 (Abrahamson [2]), gradually spreading westward. In the spring and summer of 1919, cases were reported throughout the Middle West (Bassoe,[3] Riggs,[4] Hammes [5]), and in October, 1919, it appeared almost simultaneously in Portland (House [6]), in Seattle and in Tacoma (Winslow [7]).

We have had an opportunity to observe twenty-seven cases, six of which occurred since the recent influenza epidemic and in which the clinical picture differed somewhat from those observed earlier.

The onset was usually gradual. Severe diffuse headaches, disappearing rapidly after the first four to ten days, occurred eleven times in our series. Asthenia, lethargy, general muscle rigidity with masklike features (the paralysis agitans syndrome, usually without the tremor), any or all three of these symptoms manifested themselves in every case in a greater or less degree. Cranial nerve palsies were observed, the third and sixth were involved most frequently, and diplopia or blurred vision was a common complaint. The seventh nerve was involved unilaterally twice and bilaterally once, the tenth nerve once and the twelfth nerve in two cases. Eight cases gave no evidence of cranial nerve involvement at any time during the disease.

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* Presented before the Minnesota Pathological Society, Feb. 17, 1920.

* Histopathologic Studies made in Neuropathologic Laboratory, University of Minnesota Medical School.

1. v. Economo, C.: Encephalitis lethargica, Wien. klin. Wchnschr., May 10, 1917.

2. Abrahamson, I. Prevalence of Infectious Lethargic Encephalitis, Proc. New York Neurolog. Soc., J. Nerv. & Ment. Dis. **50**:61 (July) 1919.

3. Bassoe, P.: Epidemic Encephalitis (Nona), J. A. M. A. **72**:971 (April 5) 1919.

4. Riggs, C. E.: Epidemic Lethargic Encephalitis, Minnesota Med. **3**:49 (Feb.) 1920.

5. Hammes, E. M.: Lethargic Encephalitis (with Report of Twelve Cases), Minnesota Med. **3**:118 (March) 1920.

6. House, W.: Epidemic (Lethargic) Encephalitis, J. A. M. A. **74**:372 (Feb. 7) 1920.

7. Winslow, K.: Epidemic Lethargic Encephalitis (Nona) in Seattle, Northwest Med. **18**:209 (Oct.) 1919.

In seven cases the pupils did not respond to light or accommodation; in two the pupils were miotic, in one mydriatic, and in three irregular in size.

Partial atrophy of the hand muscles (right) was observed in one case; three cases manifested weakness and one definite paralysis of one arm. Tremor of variable amplitude and location occurred in five cases. Ticlike contractions of the facial or arm muscles were observed four times. Choreiform twitchings, hemiplegic and suggestive of Huntington's type, were marked in one patient. Another patient had two general convulsions in one day two weeks after the onset of the illness, followed by motor paralysis of the right arm which improved gradually.

Urinary retention, necessitating catheterization, was present five times. Involuntary urination or dribbling occurred in only two cases.

The temperature varied between normal and 103.4 F. Five patients had a normal temperature throughout, and although they presented a typical clinical picture, the disease ran an uneventful course and the patients made a satisfactory recovery.

The mental picture has been a variable one. Delusions and hallucinations occurred. Temporary disorientation and confusion were not infrequent. One patient presented the picture of a catatonic type of dementia praecox; another one developed a typical Korsakoff's psychosis; a toxic delirium was observed in four cases associated with a high temperature. Wilson [8] described a case in which typical "Witzelsucht" was manifested through the entire course of the illness. House [6] states that euphoria in a greater or less degree occurred in every one of his patients.

The deep reflexes have been either normal, increased or absent. The youngest patient was 14 years of age, and the oldest, 54. Eight of our patients were females and nineteen were males. Of the first twelve patients, six died. Of the remaining fifteen patients, all are still living. Judging from our observation, the clinical course was gradually becoming milder until the occurrence of our recent influenza epidemic (January and February, 1920). Since then the cases (six) have been of a very severe and more toxic type, although none have been fatal as yet.

In the shortest case entire recovery occurred within three weeks. The longest one in our series began in December, 1918. At present the patient is still lethargic, and has epileptiform attacks involving the left side of his face. Another case in our series began Sept. 16, 1919. The patient remained lethargic for more than two months, had a tem-

8. Wilson, S. A. K.: Epidemic Encephalitis, Lancet **2**:7 (July 6) 1918.

perature between 100 F. and 103 F. for about one month. At present, he is able to sit up, feeds himself, takes some interest in his surroundings, but it unable to walk or talk.

The laboratory findings were quite uniform in our cases. There usually was a mild leukocytosis (average, 14,000). The spinal fluid gave a mild globulin test, a lymphocytosis varying between normal and fifty-seven per c.mm. The colloidal gold curve was of no aid in the diagnosis. In some cases, the most marked changes occurred in the syphilitic zone, in others in the meningitis zone and in several there was no change in any dilution. All blood and spinal fluid cultures were negative. No animal inoculations were done.

Tucker [9] reported two cases of lethargic encephalitis in which postmortem examination revealed some involvement of the hypophysis. His opinion was based on macroscopic examination alone, as no microscopic report was given. In our three cases which came to postmortem nothing abnormal was noted in the hypophysis on gross examination, but one of our patients presented a clinical picture suggestive of pituitary gland involvement.

REPORT OF CASES

CASE 1.—A male, 24 years of age, was first seen Nov. 25, 1919. His family and personal history are unimportant.

He contracted influenza in October, 1918. One week later he became drowsy and sleepy, and a few days afterward he became unconscious. This condition continued for about ten days, after which he gradually improved. Two weeks later he had a generalized epileptic attack which recurred at irregular intervals until Dec. 9, 1919. The attacks varied from four a day to one a month. They would commence with a "feeling of faintness," then the patient would become unconscious and have a generalized convulsion, bite his tongue and have involuntary urination. When he awakened he would feel tired and fall asleep for an hour or two. He, furthermore, described periods during which he would be confused mentally. At times he also had involuntary urination. He would be quite free from this for several weeks and then it would become very annoying for a week or so and clear up again. Mentally he is somewhat sluggish.

Since his influenzal attack, he has noticed that his breasts are enlarging, that he is losing the hair in his axillae and that he has to shave only every week or ten days, where formerly it was necessary to do this every other day. He has impairment of his sexual desire. His weight fluctuates considerably; within a week he would gain or lose ten pounds without any apparent reason.

The *neurologic examination* is negative, except for a slight Romberg. The fundi are normal; fields of vision normal. The physical examination shows a well built young man, with a dozen scant hairs on his chin only, although he has not shaved for six days. The hair on his head appears to be normal. In both axillae are a few scattered hairs. His pubic hair and his sexual organs are apparently normal. His breasts are somewhat enlarged and slightly tender on pressure. A roentgenogram of the skull is negative. There is no enlargement of the sella turcica.

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9. Tucker, B. R.: Epidemic Encephalitis Lethargica or Epidemic Somnolence, or Epidemic Cerebritis with Report of Cases and Two Necropsies, J. A. M. A. **72**:1448 (May 17) 1919.

The *laboratory findings* are: hemoglobin, 80 per cent.; erythrocytes, 4,800,000; leukocytes, 7,900; differential count, normal; blood Wassermann, negative; blood pressure, 120 systolic, 70 diastolic. The spinal fluid was under normal pressure, gave a trace of globulin, two lymphocytes per c.mm., a negative Wassermann and a negative colloidal gold curve.

Under *treatment* with whole pituitary extract 2 grains three times a day, and luminal, ¾ grain, three times a day, he has been free from attacks since Dec. 9, 1919, but has noticed no other change in his condition.

Case 2.—This case was suggestive of a thalamic syndrome. A young woman, age 29, was seen in consultation with Dr. Goltz, Jan. 27, 1920. She gave a history of having had a severe chill Jan. 17, 1920, accompanied by severe headache, fever, vertigo and general malaise. Jan. 24, 1920, she complained of blurred vision, drowsiness and nervousness. She had a slight general tremor of both arms which was not present when she was seen three days later. At this time she complained of diplopia, drowsiness, stiffness in her extremities and difficulty in starting the flow of urine. Her face was masklike, she was lethargic and answered questions sluggishly. On examination, the left pupil was larger than the right, and it was irregular. There was no response to light or accommodation. There was a paralysis of the left internal rectus. The fundi were negative. The upper extremities were normal, the lower extremities rigid, all deep reflexes normal.

About February 1, she developed irregular choreiform movements of her left arm and leg. These also involved the left facial muscles and the left sternomastoid. The movements were irregular and jerky, simulating a Huntington's chorea. They were aggravated when the patient attempted to use either left extremity. Since February 15 this has become so marked in her left facial muscles that it was difficult to understand her. She protruded her tongue normally, but there was a slight tremor present. Her right side was entirely free from these movements and the posture of the right extremities was almost catatonic. Her muscle strength was good in all four extremities. The neurologic findings have remained the same.

The laboratory findings were: leukocytosis, 14,000; negative blood Wassermann; spinal fluid under normal pressure, gave a heavy trace of globulin, fifty-seven lymphocytes per c.mm. and a negative Wassermann. Urine negative.

The lethargic condition had entirely subsided by February 24, but the choreiform movements were marked and the patient complained of difficulty in going to sleep because of them. She had a temperature of 101 F. during the first week, which gradually became normal and has remained so. She has had difficulty in taking sufficient food and is gradually losing weight and strength.

Howe [10] describes very completely a thalamic syndrome in one of his cases of lethargic encephalitis.

Six cases of lethargic encephalitis seen during the past month have presented an onset somewhat different from the previous cases. There was more evidence of meningeal irritation and mental confusion. The following case was seen Feb. 16, 1920, in consultation with Dr. Sanford at Farmington, Minn.

Case 3.—The patient was a farmer, age 52, with a negative family and personal history, except that he had an attack of pneumonia in November, 1918. About Feb. 2, 1920, he noticed a mild left conjunctivitis with severe pain in the eyeball. This gradually subsided, and within a few days he developed severe

10. Howe, H. S.: Thalamic Syndrome in Epidemic Encephalitis, Neurolog. Bull., N. Y. **2**:190 (May) 1919.

neuralgic pains in both occipital nerves. Soon after, this same condition extended into both arms, the pain being so severe that it was necessary to resort to morphin for relief. Two days later, he complained of similar pains in his lower extremities. These attacks of pain occurred frequently during the day and night. In the intervals the patient was quite comfortable.

About this time he became confused and disoriented, and during the night, he became quite delirious. His temperature was normal during the first week, but gradually increased until it was 102 F. at the end of two weeks. He also complained of blurred vision, and that if he looked to the right, the wall appeared at an angle and the pictures on the wall seemed to be at the foot of the bed. On the evening of February 15, he became drowsy and the next morning he was very lethargic, but could be aroused readily.

The *neurologic examination* was negative throughout.

The spinal fluid was under normal pressure, gave a trace of globulin, seven lymphocytes per c.mm., a negative Wassermann and a colloidal gold curve 1344210000. Since then he has developed a typical picture of lethargic encephalitis; his neuralgic pains have subsided, but he is still confused and delirious at night.

Our histopathologic studies have been based on the following three cases:[11]

Case 4 (147).—Male, age 53, admitted Oct. 16, 1919, to the Neurologic Service, City and County Hospital, St. Paul, gave a negative family and personal history. Oct. 12, 1919, the patient complained of an acute coryza, severe headache, general malaise and joint pains. He began to wander around his home in an aimless manner. The following day he became drowsy and sluggish mentally, and soon after he became stuporous. He was disoriented, but answered all other questions intelligently. He was lethargic but could be aroused readily.

The *neurologic examination* showed the following: Pupils were moderately dilated and did not respond to light or accommodation. There was weakness of the right external and left internal rectus eye muscles. The fundi were negative. The face was masklike, but the patient could move all the facial muscles. He protruded his tongue with difficulty. The upper extremities were normal, but mildly spastic. The lower extremities showed absent knee and Achilles jerks, no Babinski, no spasticity.

Leukocytosis, 11,850, blood culture negative, urine normal, except for a trace of albumin. Blood Wassermann negative. Spinal fluid gave increased pressure, a trace of globulin, fifteen lymphocytes per c.mm., a negative Wassermann and a colloidal gold curve 2334300000. Repeated lumbar punctures gave similar spinal fluid findings. The patient progressively grew worse and died eighteen days after the onset of the illness.

Case 5 (149) is of interest because of the involvement of the respiratory centers early in the disease. Male, age 45, admitted on the Neurologic Service, City and County Hospital, St. Paul, Oct. 29, 1919. His family and personal history were negative. October 15, the patient noticed that he felt weak, drowsy and wanted to close his eyes. He continued his work as a teamster for three days, until he went to sleep while driving his truck; fell down and injured his knee. He remained home for eleven days during which he "slept" most of the time. He also noticed difficulty in breathing at times. Patient was examined October 20; he was in bed motionless and with eyes closed. He could be aroused readily, answered questions, got up and walked around, and presented the typical gait and posture of a paralysis agitans. Occasionally, he would have

11. These three cases have been previously reported from the clinical standpoint by one of us (E. M. H.) in Minnesota Med. **3**:145 (March) 1920.

a general coarse tremor. At irregular intervals, he would get attacks of cogwheel respiration of from one to two minutes' duration, during which his respiration would go up to 72 per minute. He was markedly rigid.

Physical examination was negative, except for an atrophy of the left calf muscles, which patient stated he had had all his life. His pupils were normal. The light and accommodation reactions were sluggish and disappeared after three days. There was a paralysis of the right internal rectus. The face was masklike, but all movements were normal. All other cranial nerves were normal. All deep reflexes were normal, except for an absence of the left Achilles jerk.

Blood culture was negative, Widal negative, Wassermann negative; leukocytes, 12,200. Spinal fluid was under pressure, contained ten lymphocytes per c.mm., and a trace of globulin, but was otherwise normal. His temperature varied between 100 and 102 F.; his pulse was around 130; his respirations varied between normal and 72. His stupor gradually deepened and he died Nov. 9, 1919.

Case 6 (151).—Male, age 23, was admitted on Dr. A. Hoff's medical service, City and County Hospital, St. Paul, Nov. 16, 1919, with a diagnosis of suspected typhoid. A lethargic encephalitis was suggested, and Dr. Hoff kindly permitted one of us (E. M. H.) to see the patient. His family and personal history were negative. About November 9, he began to feel drowsy and weak. Soon after he had two severe attacks of epistaxis. About November 12, he developed marked photophobia, also a slight general muscle rigidity and he became stuporous. We first saw the patient November 18; he was drowsy but could readily be aroused, and he answered questions coherently. His mental condition was normal. He could not open his eyes because of marked photophobia.

Neurologic examination showed the following: Pupils equal and responded to light and accommodation. He had a paresis of the left internal rectus. All other cranial nerves were normal. All deep reflexes were normal. He had a positive right Babinski.

Blood culture was negative, Widal negative, Wassermann negative; leukocytosis, 17,000; urine normal. Spinal fluid was normal, except for a positive globulin and fifty-four lymphocytes per c.mm. November 19, he seemed confused and disoriented and developed a typical mental picture of a Korsakoff's psychosis. He died Nov. 21, 1919.

PATHOLOGY

The pathologic findings in the three cases we have studied microscopically correspond in the main to the other cases described in the literature (Bassoe,[3] Bassoe and Hassin,[12] Wegeforth and Ayer,[13] Neal,[14] Calhoun,[15] special article in *Journal American Medical Asso-*

12. Bassoe, P., and Hassin, G. B.: A Contribution to the Histopathology of Epidemic ("Lethargic") Encephalitis, Arch. Neurol. & Psychiat. **2**:24 (July) 1919.

13. Wegeforth, P., and Ayer, J. B.: Encephalitis Lethargica, J. A. M. A. **73**:5 (July 5) 1919.

14. Neal, J. B.: Lethargic Encephalitis, Arch. Neurol. & Psychiat. **2**:271 (Sept.) 1919.

15. Calhoun, H. A.: Histopathology of brain and Spinal Cord in a Case Presenting a Postinfluenzal Lethargic Encephalitis Syndrome, Arch. Neurol. & Psychiat. **3**:1 (Jan.) 1920.

ciation,[16] Marinesco,[17] Netter,[18] von Economo,[19] and Vaughan[20]). Our findings consist in congestion, edema, petechial hemorrhages, pigmentation, perivascular and diffuse infiltration of round cells, proliferative changes in the endothelial and interstitial tissues, and degenerative changes in the nerve cells and myelin sheaths. The whole central nervous system, including the meninges, shows evidence of involvement in the disease process, but the most marked changes are uniformly in the lenticulo-striate complex, the midbrain, pons and medulla. No extensive review of the literature on the pathology of lethargic encephalitis is contemplated in this report, but differences noted in our cases as compared with some of the others reported will be brought out in the description of the histopathology.

At the time of necropsy no gross lesions were noted in the central nervous system, aside from the fact that the meningeal vessels were markedly congested in Case 151 and moderately congested in Case 149. No recognizable hemorrhages or areas of softening were encountered. No pathologic changes having any significance in connection with this report were noted in other parts of the body. The postmortem examinations were performed by Dr. Kramer, pathologist at the City and County Hospital of St. Paul. The brains and cords (no cord received from Case 147) were delivered to us in 10 per cent. liquor formaldehydi.

Blocks of tissue were taken from various areas of the cerebral and cerebellar cortices, the basal ganglia, midbrain, pons, medulla and cord. Sections from these blocks were stained with thionin, Weigert's myelin sheath stain, Marchi's stain, Bielschowsky's stain, sudan III, stains for hemosiderin, and Dominici's stain (toluidin, Orange G, and eosin—an especially good stain for the study of free cells in sectioned material).

For the most part, aside from congestion of the vessels, the meninges show only very mild involvement in the inflammation. Occasional areas are to be seen in which there is a slight increase in the number of nuclei in the meninges, due to an infiltration of small round cells of the same type as will be described later in the brain substance. In one case (151), the sections passing through the attach-

16. Special Article: Epidemic or Lethargic Encephalitis (Nona), J. A. M. A. **72**:794 (March 15) 1919.

17. Marinesco, G.: Contribution à l'étude de l'histologie pathologique de l'encéphalite léthargique, Bull. de l'Acad. de méd., Par. **80**:411 (Nov. 5) 1918.

18. Netter, A.: L'encéphalite léthargique épidémique, Bull. de l'Acad. de méd., Par. **79**:337 (May 7) 1918.

19. v. Economo, C.: Wien. klin. Wchnschr. **31**:850 (July 25) 1918.

20. Vaughan, V. C.: Encephalitis Lethargica, J. Lab. & Clin. M. **4**:381 (April) 1919.

ment of the trigeminal nerve show marked perivascular and diffuse infiltration of these and other cells together with hemorrhage into the subarachnoid spaces in the region of the fifth nerve root. The changes at this point involve not only the meninges, but also the nerve trunk itself and the underlying region of the pons (Fig. 1, B and C). This area presents a decided departure from the picture of the meninges seen in any of the other sections from our cases. It appears that the meninges are even less involved in our cases than in the majority of those reported in the literature.

In the cerebral cortex of all three cases there is marked congestion of the vessels. There is slight diffuse and perivascular infiltration of small round cells (lymphocytes and plasma cells) with an evident tendency for these cells to collect especially about the bases of the pyramidal cells (Fig. 2, B). While examples of satellitosis are fairly common, no true neuronophagia is to be seen; that is, there is no evidence of actual destruction and phagocytosis of the cortical cells by the satellite cells, none of these cells are intracellular within the nerve cell bodies, the cortical cells are in a state of good preservation, showing only mild chromatolytic changes. No hemorrhages are seen within the cortical layers. No proliferative glial or endothelial changes have been noted.

The cerebellar cortices of Cases 147 and 149 appear quite normal except for a rather marked congestion of all the vessels. In Case 151, however, the molecular layer of the cerebellar cortex shows a decided diffuse infiltration of small round cells (lymphocytes) along with the congestion. Deep in the sulci of the cerebellum in this case the vessels are surrounded by scattered round cells. There are no noteworthy changes in the cells of Purkinje; our cases do not confirm the changes described by Calhoun[15] in regard to these cells. The granular cell layer appears normal in each of the three brains.

The thalamus, caudate nucleus, globus pallidus, putamen, midbrain, pons and medulla all show great uniformity in their pathology in all of our cases; hence they can be discussed together.

Perivascular infiltration of round cells about the congested vessels is the most evident change noted. These cells have invaded the walls of the vessels to a very marked extent, leaving the perivascular space of His relatively free, however (Fig. 3, A). Diffuse infiltration of these cells into the surrounding tissues is also marked. On high power examination it is seen that the majority of these cells are slightly larger than erythrocytes, and are made up of a rounded nucleus containing relatively large masses of chromatin arranged more or less wheel-like around the inside of a definite nuclear membrane, and a small amount

of, or no visible cytoplasm (Fig. 4, A). It is probable that these cells should be classified as small lymphocytes rather than plasma cells, as Bassoe and Hassin [12] have suggested. That there are plasma cells among the infiltration cell types is not at all to be doubted, however, for we have found a number of cells with relatively large amounts of cytoplasm, eccentric, rounded nuclei containing chromatin granules with a very definite wheellike arrangement and often a vacuole or vacuoles bordering on the nucleus (Fig. 4, A and B, pl). Plasma cells in our cases are distinctly in the minority, being very scarce perivascularly, and only occasionally found scattered in the tissues.

Another type of cell making up a part of the infiltration, much less numerous, however, than either the lymphocytes or plasma cells and most in evidence in the area of infiltration and hemorrhage around the origin of the fifth nerve in Case 151, is that which might be called a macrocyte, an endothelial cell or a large mononuclear cell, depending on who might be describing it. This cell is considerably larger than the lymphocytes, contains a large, rather irregular (sometimes spherical) nucleus whose chromatin material is not very dense and does not tend towards any particular arrangement about the nuclear membrane which is more delicate than that of the lymphocytes or plasma cells. The cytoplasm stains purplish (neutrophilic) with Dominici's stain and is rather granular and sometimes vacuolated. These cells are certainly phagocytic for many of them contain nuclear and other cell debris (Fig. 4, A, and Fig. 1, B). Polymorphonuclear leukocytes are absent, except in such places as contain hemorrhages; here they are not present in any great numbers and probably have simply escaped from the blood stream along with the extravasated erythrocytes. The lymphocytes and plasma cells are situated not only perivascularly, but have also wandered out into the brain substances so that in the affected areas a low power examination gives one the impression of a very marked increase in the number of nuclei in the field (Fig. 3, A).

In the basal ganglia of one case (151) is a small abscess large enough to be seen in the stained section with the naked eye, consisting almost exclusively of lymphocytes. The tissues surrounding it are infiltrated with lymphocytes and are definitely fragmented. Fortunately, we obtained practically serial sections through this region which show that the abscess has not been confused with perivascular infiltration as no vessels larger than precapillaries are in that vicinity (Fig. 3, C).

Hemorrhage is not a marked feature of the pathology in any portions of the central nervous system we have studied. Small hemorrhages, or, at least, areas in which red blood corpuscles are to be seen

in the perivascular space of His are not at all uncommon in the regions in which pathologic changes are marked — basal ganglia, midbrain, pons and medulla — and are altogether absent elsewhere (Fig. 3, B). Neal [14] states that "Frequent small and occasional large extravasations of blood are seen anywhere, but especially in the gray matter." In view of the evident vascular injury it would certainly be easy to understand how sizable hemorrhages could take place, but none of noteworthy size have been observed in any of our sections. Such hemorrhages as we have seen must have occurred shortly before death as the erythrocytes are in a state of good preservation and there is no blood pigment out in the tissues; stains for hemosiderin are negative.

Separation of the tissues of the brain in the more severely injured areas, giving the appearance of much dilated tissue spaces, is a constant feature of our sections. This looseness of the tissue meshwork is taken by us to be evidence of a marked edema, though the possibility of artifacts has been constantly kept in mind. The occurrence of this change in the affected areas and its absence in the relatively normal areas makes us feel safe in stating that there is edema present (Fig. 3, A; compare Fig. 4 and Fig. 2, A, with Fig. 2, B and C).

Glial proliferation in the affected regions is not marked at first glance. As the slides are studied, however, it appears that there is a definite increase in the number of glial nuclei. Furthermore, young types of glial cells are not uncommon with their relatively large nuclei and definitely increased cytoplasm. In one section (Fig. 4, C) an undoubted example of mitosis in a glial cell is present. No free cells of definitely glial origin have been found. Proliferative changes in the endothelial cells are also not marked in the central nervous system with the exception of the region of the meninges about the origin of the fifth nerve in Case 151. Here one finds numerous vessels slightly larger than capillaries in which the endothelium is made up of cells with marked increase in their cytoplasm and large rounded or oval, usually vesicular nuclei. Since the nucleus and cytoplasm of these endothelial cells simulate very closely the appearances in the macrocytes, and since the macrocytes are definitely phagocytic, we feel that these large free cells are undoubtedly endothelial in origin (Fig. 1, B).

Various changes are noted in the nerve cell bodies located within the severely affected areas. In these regions it is exceptional to find a normal neuron; they all show evidences of one or another type of degeneration. Examples of satellitosis about the nerve cells are very common, those of actual neuronophagia considerably less frequent, though present (Fig. 2, B and C). The satellite cells are indistinguishable in their morphology from the lymphocytes described above. Chromatolysis is the rule in these localities, usually evidenced by loss

of Nissl bodies around the nucleus and collection of the remaining tigroid substance about the periphery of the cell (Fig. 2, A). The nucleus of the cell may be relatively clear and swollen or it may be somewhat darker and shrunken. Some of the nerve cells are vacuolated (Fig. 2, A); they are seldom actually fragmented. Most of them show an apparent, if not an actual decrease in the number of their processes. Pigmentation of the nerve cells is a marked feature of all the affected areas. The pigment is extremely abundant in some of the cells, completely filling the cell body and even obscuring the nucleus. The pigment granules are small, rounded, regular and yellowish brown in all the sections excepting those stained by Sudan III when they take the red color of the stain; therefore, they are lipochrome granules. The pigment is located not only within the neurons but occurs free in the interstitial tissues and also within the cytoplasm of the glial cells (Fig. 4, B). Stains for hemosiderin are negative even in the presence of hemorrhages; hence this pigment is not a derivative of the blood pigments.

Weigert sections show no involvement of the long tracts in the brain and cord. In the nuclei within the affected areas, however, the finer myelinated fibers show definite evidences of degeneration. Some of the myelin sheaths are actually fragmented; others show the peculiar swelling and knobbing characteristic of early myelin sheath changes. These changes are particularly noticeable within the nucleus of the third nerve (Fig. 1, A), but are found even in the projection fibers of the cerebral cortex.

The spinal cord is involved to the extent of considerable congestion, some increased pigmentation of the nerve cell bodies and fairly common examples of chromatolysis.

There are no changes of any significance in the ependyme of the ventricles of the brain or the central canal of the cord.

It is worthy of mention that in the rootlet of the trigeminal nerve in Case 151 there is very marked perivascular and some diffuse infiltration of lymphocytes (Fig. 1, C). We have seen no inflammatory changes in any of the other nerve rootlets.

DISCUSSION

The laboratory findings in our cases have thrown no light on the etiology of lethargic encephalitis. This is in accord with most investigators. v. Wiesner[21] cultured a gram-positive diplococcus from a monkey which had previously been inoculated subdurally by an emul-

21. v. Wiesner, P. R.: Die Aetiologie der Encephalitis lethargica, Wien. klin. Wchnschr. **30**:933, 1917.

sion of the brain and cord from one of v. Economo's patients. Strauss, Hirshfeld and Loewe [22] also successfully inoculated monkeys with an emulsion of the human brain and produced the characteristic lesions of lethargic encephalitis. In a later report they state that they have isolated a gram-positive organism resembling that described by Flexner and Noguchi in poliomyelitis.

Although no definite relationship has been established between influenza and lethargic encephalitis, the clinical evidence is very suggestive for every epidemic of lethargic encephalitis has been preceded by a pandemic of influenza.

Neal,[14] Bassoe and Hassin,[12] and Calhoun [15] have pointed out the similarity pathologically between lethargic encephalitis, acute anterior poliomyelitis and trypanosomiasis, and have considered the possibility of a common etiology, but have been able to reach no definite conclusions.

SUMMARY AND CONCLUSIONS

1. No definite etiologic factor has yet been established in lethargic encephalitis. The relationship clinically to influenza is very suggestive.

2. Although the general symptomatology is profound, the localizing symptoms are not so marked as the widespread pathologic findings would lead one to expect.

3. Asthenia, lethargy, muscle rigidity and cranial nerve involvement are the outstanding features of lethargic encephalitis clinically.

4. Pathologically, the picture is an inflammation of the brain and cord characterized by a perivascular and diffuse infiltration of lymphocytes especially in the basal nuclei and gray matter of the brain stem.

We wish to express our appreciation for criticism and advice cheerfully given by Drs. A. S. Hamilton and C. E. Nixon in the preparation of this paper; also, credit is due Miss Margaret Graham for her intelligent interest in preparing the tissues on which our pathology is based.

22. Strauss, I., Hirshfeld, S., and Loewe, L.: Studies in Epidemic Encephalitis (Encephalitis Lethargica), New York M. J. **109**:722 (May 3) 1919.

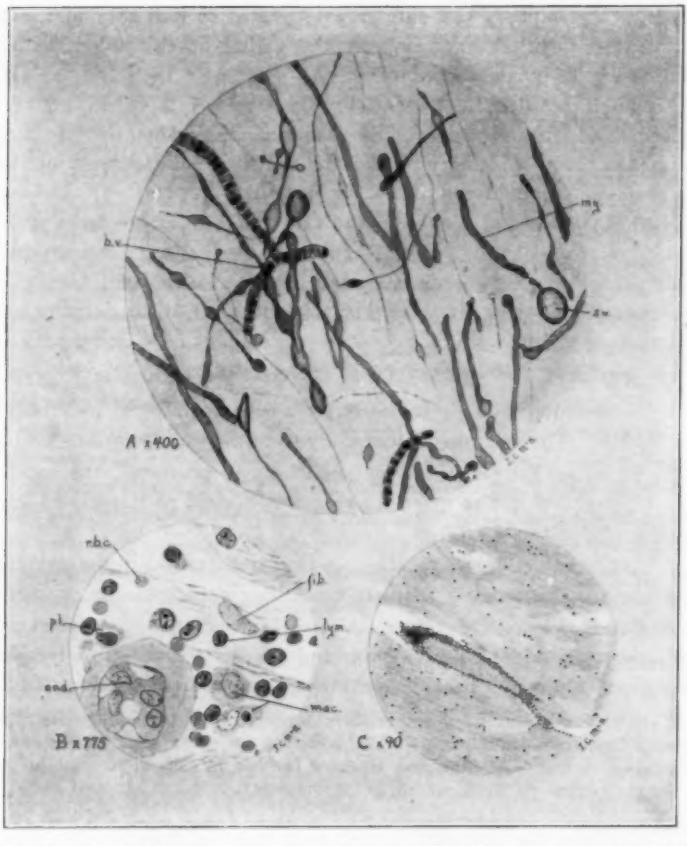

Fig. 1.—A. × 400. Nucleus of oculomotor nerve in Case 151 stained with Weigert's myelin sheath stain. Knobbing and fragmentation of the myelin sheaths. b. v., blood vessels; my., myelin sheath fragmented; sw., swelling or knobbing of the same sheath. B. × 775. Meninges near the attachment of the fifth cranial nerve in Case 151. Swollen endothelial cells still attached to a vessel wall, apparently ready to break loose and give rise to macrocytes. Hemorrhage and infiltration. end., swollen endothelial cells; fib., fibroblast nucleus; lym., lymphocyte; mac., macrocytes, one of them containing cell debris; pl., plasma cell; r.b.c., erythrocytes in the subarachnoid spaces. C. × 90. Perivascular infiltration in the root of the fifth nerve in Case 151.

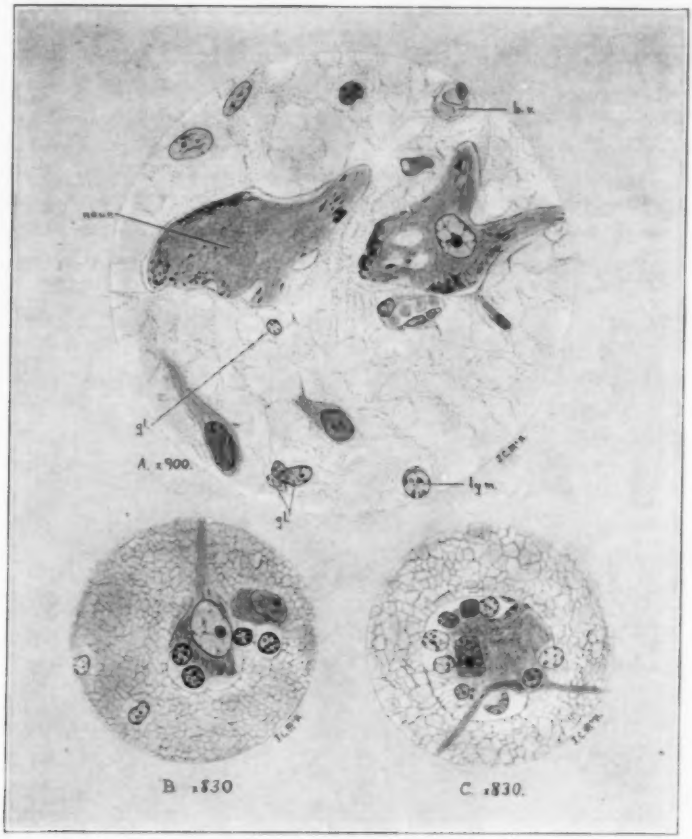

Fig. 2.—A. × 900. Basal ganglia from Case 149. Chromatolytic, swollen nerve cells, one of which is vacuolated. b.v., blood vessel; gl., glial cell; lym., lymphocyte; neur., neuron. B. × 830. Cortex from Case 147. Satellitosis about the bases of two pyramidal cells. C. × 830. Basal ganglia from Case 151. Neuronophagia. Chromatolytic ganglion cells with pyknotic nucleus.

Fig. 3.—A. × 75. Midbrain from Case 147. Perivascular and diffuse infiltration of lymphocytes. Marked edema. Satellitosis about the nerve cell bodies. b.v., blood vessel; neur., neurons with satellite cells. B. × 360. Basal ganglia from Case 147. Small hemorrhage about a vessel. b.v., blood vessel; r.b.c., extravasated erythrocytes in perivascular space of His. C. × 75. Basal ganglia from Case 151. Small abscess made up of lymphocytes. Fragmentation of the surrounding tissues.

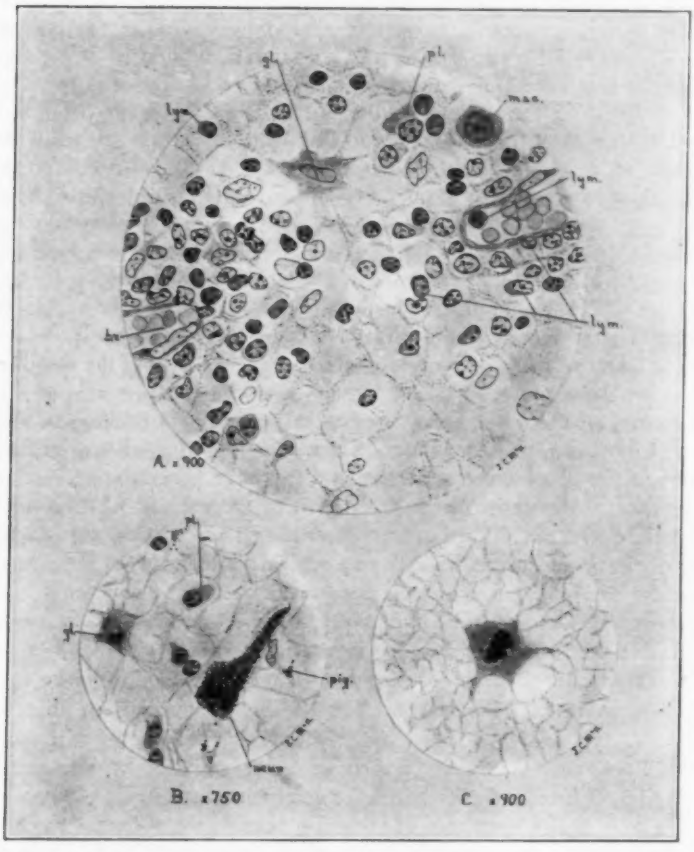

Fig. 4.—A. × 900. Pons from Case 149. Cell types making up the infiltration. b.v., blood vessel; gl., glial cell; lym., lymphocyte; mac., macrocyte; pl., plasma cell. B. × 750. Midbrain from Case 147. Pigment granules in a nerve cell body, in a glial cell and free in the tissues. gl. glial cell; neur., neuron filled with pigment; pig., pigment granules; pl., plasma cell. C. × 900. Basal ganglia from Case 151. Mitosis in a glial cell.

TRENCH FEVER*

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Disease is the accomplice of war. To the uninitiated, at first glance, it seems that the chief wastage of man power in armies is produced by weapons of the enemy; to the initiated, on the other hand, it is well known that the wounds produced by bacteria are as fruitful a source of disability as those caused by bullets. One reason for this attitude on the part of the casual observer is that the injuries of battle are more spectacular than those of disease. They are more unusual, both in their mode of production, and in the manner in which they respond to treatment. The care of the wounded often yields striking results. The humanitarian instincts, aroused by the fact that the injuries were received as a direct result of sacrifice, properly lead to the building up of an intricate organization for the care of the wounded. The problems must be solved largely in the actual presence of war. Sickness, on the other hand, is more easily tolerated because it is the lot of most men to suffer illness. The problems of disease are always with us; most of those presented by war have been studied in times of peace. Almost in direct proportion to the rate at which a given disease causes fatalities, in contradistinction to casualties, are effective prophylactic measures studied and put into effect. The antivenereal disease campaign in our own army is a striking exception to this statement. The prophylaxis of typhoid fever and smallpox was more effective than that of dysentery or the pyodermias. It may be urged that the measures for preventing the first two diseases are more easily applied in war. This is true; but had the prevalence of the fatal disease, typhus fever, been as great as that of the nonfatal, but disabling affection, trench fever, no doubt more effective measures would have been early instituted for combating it.

The complete ignorance of the existence of such a disease as trench fever, with the consequent lack of knowledge as to its mode of spread, made it impossible to apply prophylactic measures until this information was available. A very real impediment to the study of a disease during war is that the patients are rarely under the same observers or group of observers during the entire course of their illness. This was especially true during the first part of the recent war, before the lines had become stabilized and hospitals were stationed close enough to the front to permit the retaining of the sick for longer periods.

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*Lecture delivered before the Harvey Society, New York, Jan. 10, 1920.

For these reasons it was not generally recognized until the spring of 1915 that a hitherto unknown disease was fairly widespread among the combatant troops. At this time, medical officers in both the British and the German armies almost simultaneously noticed a five or six day relapsing fever in many patients who complained of severe and persistent pains in the shins. Graham,[1] Herringham[2] and others saw many examples of this condition among British troops from the trenches but not among the troops in the rear; so the name of trench fever was applied. His[3] and Werner[4] saw their first cases on the east German front in Poland and Wolhynia. The former applied the term Wolhynian fever to the disease because of the supposed source of the infection; the latter used the term five day fever because of the interval between relapses. Because of the almost simultaneous appearance of cases on the two fronts it is difficult to determine the original source of the infection; but Grätzer,[5] a battalion medical officer in the Eighty-Fourth Austrian Infantry Regiment, states that he had observed cases among soldiers under his care since the winter of 1914. He gave a very good description of the malady; and because of its increase in winter and when crowding of the soldiers was necessary, he attributed the spread to some insect vector. He saw his first cases when his regiment was on the Nida, and learned from the inhabitants of that region that a similar condition was known to them before the war. Franke[6] also states that he recognized the disease as having occurred in Lemburg in previous years, when it was known as influenza with relapses. It seems, therefore, from the evidence at hand, that the original source of the infection was somewhere in Russia, and that the disease was spread to all of the battle fronts in Europe by German and Austrian troops as they moved from one area to another.

It was early noted by some observers that an enlarged, hard spleen, often accompanied by perisplenic tenderness, could be demonstrated in many of the patients. The outstanding features of typical cases were, however, the sudden onset with marked febrile reactions, headache and general body pains, closely resembling the onset of influenza, but followed in a few days by pain and tenderness in the shins, and a spiky type of relapsing fever. There were many patients who complained of painful shins, especially at night, but in whom there was no history of an abrupt onset, or of a distinct febrile bout. Various

1. Graham, J. H. P.: Lancet **2**:703, 1915.
2. Herringham, W.: Quart. J. Med. **9**:429, 1916.
3. His, H.: Berl. klin. Wchnschr. **53**:738, 1916.
4. Werner, H.: München. med. Wchnschr. **63**:402, 1916.
5. Grätzer, A.: Wien. klin. Wchnschr. **29**:295, 1916.
6. Franke, M.: Wien. klin. Wchnschr. **30**:45, 1917.

explanations, such as long marches, flat feet, standing in mud and water, rheumatism or myalgia were suggested to account for these unusual pictures. In still other patients with similar symptoms, the pyrexia was continuous, resembling that of typhoid or paratyphoid fever, or intermittent like that of sepsis.

Much confusion in diagnosis arose from the presence of so many patients having certain symptoms in common, yet presenting, on the other hand, so many individual variations. In fact, it was so difficult to establish a normal or typical picture of the disease that the terms P. U. O. (pyrexia of undetermined origin) or influenza were applied in many instances.

The ignorance of the existence of atypical forms of trench fever, the practical impossibility in many cases of making a positive diagnosis, and the great difficulty in establishing adequate sanitary measures during war of movement, were all factors that resulted in the infection being widespread before the disease had become recognized as a separate entity. A serious study to determine its nature was soon undertaken by investigators on both sides of the line. The first extensive report was that of McNee, Brunt and Renshaw,[7] who used as experimental subjects British soldiers who had volunteered for inoculation. These workers showed that the virus of the disease was contained in the whole blood and that it could be transferred from man to man by intravenous or intramuscular injection. In their experiments the plasma or serum obtained from infectious blood did not contain active virus unless hemoglobin tinged. The red blood cells, on the other hand, were infectious, even after five washings. Material known to contain virus was not infectious after passage through a Berkefeld filter; but in spite of this fact these workers were unable to demonstrate microscopically any micro-organism. This work, unfortunately, was interrupted before it was shown conclusively that trench fever was not a modified form of typhoid or paratyphoid fever, and before it had been established that the infection was transmitted by some insect vector. In spite of the questions that were left undecided, the observations of McNee, Brunt and Renshaw stand as the first important contribution to the nature of this disease. They showed that the inoculation of blood from a patient with the short form of fever, might result in infection showing a prolonged course, with spiky relapses every fifth day; the essential unity of a disease with multiform manifestations was therefore established.

In the year following this work, there were many unconfirmed reports from German workers, that they had been able to transmit

7. McNee, J. W., Brunt, A., and Renshaw, E. H.: Brit. M. J. **1**:295, 1916.

the infection to animals. Jungmann and Kuczynski [8] claimed to have produced a fatal infection in mice; and Strisower [9] reported that both cats and mice succumbed to a fatal infection following inoculation with blood from trench fever patients. The animals did not present a clinical picture similar to the disease in man; nor were they successfully inoculated through several generations. Most workers agree, however, that it is impossible to transmit the typical disease to the ordinary laboratory animals; although extensive experiments have not been attempted with the higher apes. For this reason, all experimenters have found it necessary to resort to the inoculation of human volunteers in order to obtain any definite, useful information in reference to the nature and mode of transmission of the infection. Werner and Benzler [10] successfully inoculated themselves by means of instramuscular injections of blood from trench fever patients. Six months later Werner allowed himself to be bitten by lice that had previously fed on trench fever patients, and after an incubation period of eight weeks developed a mild illness that was diagnosed as Wolhynian fever. Kuczynski [11] also contracted the disease after being bitten by supposedly infected lice. In all of the experiments carried on by the German workers there is no mention that the inoculated subjects had been isolated from other cases of trench fever, or that special efforts were made to elimiate other sources of infection. It is a well known fact that doctors, nurses and attendants in military hospitals where the disease was prevalent were specially subject to accidental infection. Furthermore, there is little evidence to show that the stocks of lice were free from infection before use in the experiments. Davies and Weldon,[12] of the English army, allowed themselves to be bitten by lice immediately after an infecting feed on trench fever patients; the lice were originally collected from soldiers. One of them developed trench fever twelve days later. The same criticism that has just been made of the German work can equally well be applied to their experiment. It is not intended to underestimate the efforts of any of these workers, but rather to point out that the results were not sufficiently conclusive to warrant the undertaking of extensive prophylactic measures along any particular line.

Late in 1917, Pappenheimer and Mueller [13] of the Presbyterian Base Hospital of New York succeeded in transmitting the disease to one

8. Jungmann, P., and Kuczynski, M. H.: Deutsch. med. Wchnschr. **64**: 359, 1917.

9. Strisower, R.: München. med. Wchnschr. **65**:476, 1918.

10. Werner, H., and Benzler, F.: München. med. Wchnschr. **64**:695, 1917.

11. Kuczynski, M. H.: Reported in Jungmann, P., Monograph, loc. cit.

12. Davies, F. C., and Weldon, R. P.: Lancet **1**:183, 1917.

13. Pappenheimer, A. M., and Mueller, J. H.: Reported in American Red Cross Committee Report, loc. cit.

of three volunteers by allowing lice to feed, first, on several trench fever patients and then on the volunteers. In their experiments, the volunteers were kept carefully isolated. Unfortunately, the patient to whom the disease was transmitted suffered from a complicating femoral phlebitis. I saw him on several occasions, and have little doubt that he had true trench fever. Here again, it could not be asserted positively that the lice were not infected before they fed on the trench fever patients.

These isolated observations were all highly suggestive, but awaited confirmation by more extensive experiments before they could be translated into active effort. In fact, there were negative experiments, such as those recorded by Sundell and Nankivell,[14] and many reports of patients who denied having been bitten by lice, that threw doubt on the louse transmission hypothesis. Epidemiologic studies were highly unsatisfactory because of the constantly changing population in most military units. The proof that rats were the active agents in the transmission of spirochetal jaundice naturally suggested that trench fever might be spread in a similar manner. It is evident that it was impossible to frame any effective program to combat the spread of the disease in the face of so much conflicting evidence and opinion.

In the meantime accumulated evidence showed that trench fever was one of the largest sources of wastage of man power in the fighting armies. It is estimated that during 1917 and 1918, before the influenza epidemic, it was the cause of from one-fifth to one-third of all of the cases of illness in the British armies in France. The German reports indicate that this disease was responsible for at least one-fifth of the illness in the armies of the Central Powers. Although the illness was never fatal, it resulted in prolonged disability. A report from the Boulogne Base [15] of the British army shows that patients with trench fever were unfit for military duty on an average of from sixty to seventy days, and that in addition at least 10 per cent. of them became semipermanent invalids.

This is a brief sketch of the state of our knowledge at the time of the formation of two commissions to study the disease more carefully. It was perfectly evident that to obtain the most accurate information it was necessary that the experimenters should be free from the manifold duties of an army medical officer. The conditions under which members of these commissions worked satisfied these requirements. The British Commission in London had the advantage of a permanent hospital and laboratories, and the assistance of the workers of the Lister Institute. Volunteers for inoculation and fresh

14. Sundell, C. E., and Nankivell, A. T.: Lancet **1**:399, 1918.

15. Elliott, T. R., Lewis, D. S., Thursfield, J. H., Jex-Blake, A. J., and Foster, M.: Lancet **1**:1060, 1919.

cases of the disease were not so available as in France. It was possible to conduct the experiments in a more leisurely manner, and thus to study in more detail the problems as they arose.

The commission [16] formed under the auspices of the American Red Cross, on the other hand, started its work under field conditions. Later it was necessary to move the laboratory, patients and personnel to Paris, where better facilities were available. We were compelled to solve the problem as quickly as possible. We had the advantage of starting the work in a place where numerous examples of fresh infection of the disease were available, and where the Trench Fever Commission of the British Expeditionary Force had been carrying on clinical studies for some time. The advice of experienced observers was, therefore, available both in the selection of suitable patients from which to obtain the virus, and in the decisions as to the nature of the disease produced. Every facility at their disposal was offered by both the British and American military and medical authorities. The members of the commission were chosen because they could carry out their particular part of the work with the least possible delay. Captain Peacock was loaned by the R. A. M. C. to help in the entomologic work, because of his previous experience with the life and habits of insects.

These details are related in order to make clear the conditions under which the answers to the various problems were obtained. Throughout the entire period of our work there was the closest cooperation between all of the interested organizations. As fast as positive results were obtained by one commission they were made known to the other, and thus much time was saved to both. It is only natural that different lines of investigation should have been followed by different workers, and somewhat divergent results obtained; but in the main facts the two commissions agree. This communication, therefore, will consist largely of a résumé of the work of these two bodies.

Before proceeding, however, it is well to credit the volunteers for the large part they played in the success of the experiments. Probably in the investigation of no other disease have so many men submitted themselves to artificial inoculation. Even though they could be reasonably sure that their illness would not be fatal, they knew that they would suffer much pain and incapacity for an indefinite period. In spite of this knowledge, both our own soldier volunteers, and the British civilians who offered themselves, underwent the trying experience with the greatest fortitude. As a direct result of their sacrifice,

16. The Commission consisted of Majors Strong and Opie, Captains Macneal, Baetjer and Pappenheimer, Lieutenant Rapport and myself, all members of the Medical Corps of the U. S. Army, and Captain Peacock, an entomologist from the Royal Army Medical Corps.

much information was obtained that led to the institution of measures for the prevention of the spread of trench fever in both military and civilian population.

It will be recalled that although McNee, Brunt and Renshaw [17] had fairly well proven, by human transmission experiments, that trench fever was a disease entity related in no way to the typhoid fever group, there were many clinicians who still held that the malady was a form of enteric fever modified by the immunity that had been induced in soldiers by protective inoculations. There were many cases in the British enteric fever hospitals diagnosed by means of agglutination reactions as typhoid or paratyphoid fever that presented clinical pictures resembling trench fever in almost every respect. One of our first problems was, therefore, to confirm McNee's observations, and to establish definitely that the patients with whom we were working were not suffering from any other disease than trench fever. More than thirty volunteers [18] were inoculated with blood or some fraction of blood. In all of the original patients from whom the virus was obtained, as well as in those that developed the disease as a result of inoculation, it was proven by bacteriologic examination that no known bacterium played any etiologic rôle in the condition under consideration. Like McNee and his co-workers, we found that the whole blood contained the virus, but in contradistinction to their findings, we determined that the plasma, obtained from citrated blood, was always infectious. In four out of five experiments the incubation period in the patient inoculated with citrated plasma was shorter than in control patients inoculated with the whole blood. It seemed, therefore, that the plasma contained the virus in greater concentration. Clear serum, obtained by centrifugalizing coagulated blood, no longer contained active virus. A similar result with serum was recorded by the British Commission.[19] It seems, therefore, that the virus is either enmeshed in the fibrin network of the blood clot, or is destroyed by some substance set free during clotting. The incubation period in patients inoculated with citrated blood after standing outside of the body for two or three hours, was longer than in those patients that were inoculated immediately.

In connection with the problem of immunity in trench fever it is interesting to note that the injection of blood obtained from patients on the first to the fourth days of the disease resulted in positive

17. McNee, J. W., Brunt, A., and Renshaw, E. H., loc. cit.

18. Trench Fever, Report of Commission of American Red Cross Committee, Oxford Press, London, 1918.

19. Trench Fever, W. Byam and Others: Report of British War Office Trench Fever Investigation Committee, Oxford Press, London, 1919.

infections with an incubation period of from five to seven days; while in those that were inoculated with blood obtained on the sixth or seventh day, the incubation period was from thirteen to twenty days. Furthermore, a susceptible subject injected with blood from a patient during an active relapse on the eighty-second day, did not contract trench fever. Later experiments make it reasonably certain that this blood contained virus. This evidence points to the development of immune bodies in the serum of patients as the disease progresses; such immunity explains to a certain extent the mechanism of recovery.

Our attempts to pass the virus in the plasma through a Berkefeld filter met with failure, as did the filtration experiments of McNee and his co-workers. One patient, who was inoculated with the filtrate of crushed and ground infectious erythrocytes, developed symptoms and signs of the disease from the eighth to the eleventh day, but no fever until the fiftieth day after inoculation. While the results in this single experiment were suggestive, more conclusive evidence of the filterability of the virus was not forthcoming until later, and hence will not be discussed until other evidence of the nature of the infectious agent is presented.

In none of our experiments was the virus demonstrable in the feces of trench fever patients; on the other hand, it was occasionally present in the mixed sputum and saliva. In contrast with these findings, the frequency with which the urine of trench fever patients was infectious was noteworthy. All of five subjects inoculated with unfiltered urine sediment developed the disease, although one patient was inoculated three times before positive results were obtained. The material for inoculation was prepared by centrifugalizing urine, drying the sediment to a gummy mass, and keeping it at room temperature. The combined sediment collected on different days from several patients was used in all of the experiments. Here again, the inoculation was effected by applying the material to lightly scarified skin. This series of experiments demonstrated somewhat the resistant nature of the virus, in that the high salt concentration that resulted from evaporation of urine, was sufficient to kill most bacteria and spirochetes. There is, however, some evidence that this manipulation did decrease the virulence of the infectious material, for in four of five patients inoculated with urine sediment, the incubation period was two weeks or more. Another explanation for this longer incubation period is that the virus may have been present in the urine in not so high concentration as in the blood. In framing measures for the prevention of trench fever, these experiments indicate the necessity of considering the urine and sputum as possible, if not the chief, sources of infection.

While the foregoing experiments were an important part of our work in that they prove beyond doubt the essential nature of trench fever, and provided known sources of infection for the elucidation of other problems, they were only contributory to the main object of our commission—namely, the determination of the rôle of insects in the transmission of the disease. From the beginning it was evident that military operations might interfere with the continuation of the work. The experiments were, therefore, planned to give a positive answer in the shortest time.

All of the lice used were reared from eggs and fed on normal subjects. Altogether eleven different people served to feed these normal lice, without developing the disease. Similar findings of the British Commission should serve to quiet the contention that the symptoms of trench fever may be produced by the action of normal lice.

In order to infect the lice, they were allowed to feed on trench fever patients by means of the box method. Between feedings they were kept at about 30 C. in entomologic boxes prepared from ordinary cardboard pill boxes, and were fed two or three times a day by placing the open side of the box upon the arm of the subject for thirty minutes. After several infecting feeds, they were transferred to specially prepared cells and placed on the normal subject. These cells were designed so that the lice might live under as normal conditions as it was possible to reproduce artificially. A piece of flannel shirting was placed inside of a larger piece of calico, which was then fastened to the arm of the subject by means of adhesive tape; the arm was finally covered with cotton and enclosed in a sleeve that was fastened to the skin at the top and bottom. In this form of container the insects could feed, breed, live and die, in almost the same manner as when they infest the clothing of soldiers. The only limitation of their normal activities was that they could not migrate to other portions of the body or to other persons. It was difficult for the volunteer to scratch the skin through the several thicknesses of cloth and cotton, so that at times when the cell was removed for inspection he was allowed or encouraged to scratch. In some instances, however, there were no lesions of the skin other than those produced by the stabbers of the lice. Among twenty-three subjects who harbored infectious lice in this manner, eighteen, or 78 per cent., developed trench fever. Two others, in whom a special effort was made to prevent any skin lesion, except that resulting from the bite of the lice, also developed the disease after an incubation period of four and five weeks, respectively. In these two experiments the lice never came into direct contact with the skin of the subject, but were allowed to feed by biting through the meshes of the chiffon cover of the box in which they were kept; in the intervals between feeds the boxes of lice were put in the incubator. In the

large majority of our experiments, therefore, infectious lice living under natural or artificial conditions were able to transmit the disease to susceptible subjects. In some instances the lice were on the subject for as short a time as three days; in others for as long as thirty days. In some experiments, after the infecting feeds, the lice were transferred to successive subjects in order to eliminate possible mechanical transference of the virus. The incubation periods varied between fourteen and thirty-eight days, with an average of about twenty-one days. This long incubation period should be kept in mind in connection with the scarification experiments discussed later.

The British commission [19] was less successful in transmiting the infection by the bites of infected lice. In a total of eight experiments only two subjects developed the disease. In all of these trials, however, the lice were fed entirely by the box method, as in two of our experiments mentioned above. The difference in the method employed by the two commissions explains, no doubt, the difference in their results. The failure of the workers in London to transmit the disease by the bites alone of infected lice, led them to study the effect of applying the excreta of such lice to scarified skin. This resulted in positive infections in the large majority of their experiments. This fortunate outcome opened up a fruitful field which was explored by them with brilliant results. In demonstrating this form of inoculation they developed a method that permitted the study of the evolution of the virus in both lice and patients.

It was shown that the excrement of practically all lice that have bitten trench fever patients is infectious when applied in suitable quantities to the skin of normal individuals, either by scarification or subcutaneous injection. Volunteers could also be infected by introducing the material into the conjunctival sac, but not by insufflation into the nose or by ingestion with the food. The incubation period in the majority of the patients infected by cutaneous scarification was from seven to nine days; that from conjunctival inoculation was about twice as long.

It was established that a certain interval must elapse between the infecting feed and the excretion of actual virus by the lice. In one series of experiments when the insects were fed on a patient with trench fever on the second day of his disease, this interval was five days; in another, when the infecting feed was obtained from a patient on the seventy-ninth day of his disease, the interval was twelve days. An observation of even as great interest was the length of the incubation period in the volunteers infected with excreta passed by lice on different days after the infecting feed. For instance, the incubation period in the patients infected with excrement passed on the fifth and seventh days after the infecting meal was sixteen and thirteen days,

respectively; while in the patients infected with excrement passed from the ninth to the twelfth days, it was from seven to nine days. This evidence points to one of two hypotheses: either the virus of trench fever goes through a developmental cycle in the body of the louse, or it is taken into the body of the insects in extremely minute quantities, and there must undergo an increase before it can be excreted in sufficient quantities to infect man. The fact that within certain limits the incubation period is shortened by increasing the amount of virus most easily explains why the first virus passed by the lice is less actively infectious than that passed after the ninth day. The minute quantity of the virus that may induce an attack of the disease is shown by the fact that 0.1 mg. of excrement injected subcutaneously was infectious, while 0.05 mg. was not. The proof that after a lot of lice have been infected they continue to pass the virus during the remainder of their lives also supports the hypothesis that the virus simply increases in the body of the parasites. It was further demonstrated that a single louse may pass active virus as late as thirty-two days after it has fed on a trench fever patient.

The British commission also turned its attention to the length of time during which a trench fever patient is capable of infecting lice — in other words, as to how long the virus is circulating in the blood. It is evident that in a disease such as trench fever, in which some patients show evidence of active infection for only two days and others exhibit symptoms for two years, it would be a tremendous, if not impossible, undertaking to determine when every patient is no longer infectious. Both commissions have shown that the virus is circulating in the blood of practically all patients during the first few weeks. Byam [20] and his co-workers demonstrated that lice may abstract the virus from the blood of patients showing evidence of chronic infection as late as the three hundredth and the four hundred and forty-third day after the onset of the disease. Lice were also infected by patients during periods of intermission from active symptoms in earlier stages; these subjects usually had relapses later. Two of our patients, on the other hand, failed to infect lice that were allowed to feed on them about the hundredth day after the onset of fever. It seems that when a patient has recovered completely he is no longer a source of danger. It is difficult, however, to determine when this time has arrived, for many patients after long periods of freedom from symptoms have late relapses. It is probable that such carriers of the virus, among infested troops often served to spread the disease throughout the armies.

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20. Byam, W., and Lloyd, L.: Proc. Roy. Soc. Med. **13**:19, 1919.

Both commissions also showed that the virus is not transmitted to the offspring of infected lice through the eggs. The British commission demonstrated that pediculus capitis can transmit the infectious agent through the excreta in the same manner as does the pediculus corporis. Bed bugs, on the other hand, did not transmit the disease. Although no experiments are recorded on the transmission of the virus by other blood-sucking insects, it seems that the chief offender in the armies was the body louse.

We are now in a position to inquire into the nature of the infecting agent of trench fever. It is found in three different mediums: (1) Blood of patients; (2) urine of patients; (3) the excrement of lice that have fed on trench fever patients. Thus a variety of possibilities present themselves for consideration.

The early demonstration that the blood was infectious led many workers to search microscopically for the offending micro-organism, with widely divergent results. McNee and his collaborators were unable to find anything in blood films that could be definitely established as a micro-organism. They were also unable to infect patients with the Berkefeld filtrate of infectious blood. Our own investigations as to the filterability of the virus in the plasma also yielded negative results. On the other hand, one experiment with the filtrate of crushed, washed erythrocytes, known to be infectious, suggested that the negative results with filtrates of plasma containing virus might have been due to the blocking of the pores of the filter with the large colloid particles of globulin and albumin.

In this experiment the patient presented an atypical picture of trench fever, in that he had such symptoms as pain and tenderness in the usual locations, and an enlarged spleen from the second to the eighth week after inoculation, but no definite fever until the fiftieth day. Then, after a short bout of fever, accompanied by an increase of symptoms, the spleen diminished in size, and all of the symptoms of the preceding six weeks disappeared entirely. No other explanation for the peculiar clinical picture could be advanced except that a very small amount of the virus had passed through the filter, which had not been clogged with the plasma, because this substance had been removed in the washing of the erythrocytes.

After the demonstration of the infectivity of the urine of patients, and of the excrement of infected lice, it seemed advisable to repeat the filtration experiments with these substances, for in them there was probably the maximum quantity of virus with the minimum of admixed colloids. Two sets of experiments were, therefore, performed: The dried urine sediment collected from several patients was pooled and divided into two portions. One, without further treatment, was applied to the scarified skin of two volunteers in order to prove that the

material under consideration was infectious; the other was suspended in physiologic sodium chlorid solution and passed through an unglazed porcelain filter (Chamberland L) which held back *B. typhosus*. Two volunteers were injected intravenously with this filtrate. The controls, inoculated with the unfiltered sediment, developed mild types of trench fever, after incubation periods of fifteen and sixteen days, respectively. One of them suffered relapses; the other did not. The mildness of the symptoms induced in these controls indicates that the virus in this particular set of experiments was either attenuated or present only in minute quantities. One of the volunteers, injected with the urine filtrate, did not develop sufficiently distinct symptoms to warrant a diagnosis of trench fever; the other volunteer, inoculated with the same filtrate, developed trench fever after an incubation period of twenty-one days. In order to confirm the diagnosis, however, lice were allowed to feed on him from the fourth to the seventeenth days, and with their excrement another volunteer was inoculated by cutaneous scarification. He developed absolutely characteristic trench fever after an incubation period of nine days.

The last series of filtration experiments was carried out with the virus contained in the excrement of lice that had fed upon trench fever patients. One and one-half grams of this material was collected and divided into two portions. With one of them four volunteers were inoculated, all of whom developed trench fever after periods ranging from seven to ten days. The other portion was suspended in physiologic sodium chlorid solution so that the final strength of the suspension was 2 per cent. It was then passed through a Chamberland filter that held back *B. typhosus*. It is calculated that under the pressure conditions (760 mm. Hg) this filter would hold back any organism larger than that of pleuropneumonia. Three volunteers were inoculated intravenously with this filtrate. One remained free from symptoms. The second, after a period of five days, developed a low grade septic type of fever lasting seven or eight weeks, during the latter half of which time the pulse rate was much elevated; the spleen was intermittently palpable from the tenth to the thirty-fourth day; pain and tenderness, except headache, however, were never distinct features. In connection with this case, it may be recalled that Byam has demonstrated by inoculation experiments that a patient may have trench fever with an afebrile course throughout the entire period of observation. No other condition could be found in our patient to explain the peculiar clinical picture. The third of the volunteers, inoculated with filtrate of saline suspension of excrement, after an incubation period of twenty-one days, had an attack of trench fever with two relapses. During the first bout of fever there was an accompanying bronchitis;

but nothing except the occurrence of trench fever could explain the relapses with typical enlarged spleen, successive crops of macules, and characteristic pain and tenderness.

It seems definitely established, therefore, that the infectious agent, during at least one stage of its development, can be passed through a porcelain filter if the pores of the filter are not blocked with admixed protein. These experiments have been described in detail because the negative filtration experiments of other workers have cast a certain amount of doubt on the validity of our results. The failure of McNee, Brunt and Renshaw is easily explained. The only other details of experiments in reference to the filterability of the virus are a set of five reported by Arkwright.[21] Infected lice excrement was suspended in normal saline and subjected to filtration through either Berkefeld or Chamberland filters at different pressures. In two experiments in which the pressure was between 300 and 400 mm. of Hg, the injection of the filtrate was followed by entirely negative results; in a third, in which the pressure was between 200 and 300 mm. of Hg, the injection of the filtrate into a susceptible subject was followed in eight days by fever and abdominal pain, the causation of which was in doubt. In these three experiments the filter held back *B. prodigiosus*. In two other trials in which the pressure was between 600 and 740 mm. of Hg, and in which the filter allowed *B. prodigiosus* to pass, the injection of the filtrate was followed in one subject by typical relapsing trench fever, and in a second by no unusual symptoms at all. It is of interest to note that in the last two experiments the filtrate was collected from the same material during two successive periods. The advantage of having several subjects with which to test a given filtrate is well illustrated. A similar demonstration was afforded by our filtration experiments.

In connection with the filterability of the virus of trench fever, it may be recalled that a similar divergence in results has existed in the demonstration of the filterability of several of the filter passing viruses. Many experiments were made before it was established definitely that the virus of smallpox and vaccinia was filterable. Ricketts[22] was unable to filter the micro-organism shown by him to be contained in the blood of patients suffering from typhus fever. Both Nicolle[23] and Prowazek,[24] on the other hand, have demonstrated that the typhus fever virus, under proper conditions, will pass through a Berkefeld filter. In order to demonstrate the filterability of many unknown

21. Arkwright, J. A.: Brit. M. J. **2**:233, 1919.
22. Ricketts, H. T., and Wilder, R. M.: J. A. M. A. **54**:463 (Feb. 5) 1910.
23. Nicolle, C., Conor, H., and Conseil, E.: Compt. rend Acad. Sc. **151**:685 1910.
24. Von Prowazek, S.: Beitr. z. klin. d. Infectionskrank. u. z. Immunitätsfsch. **4**:5, 1915.

viruses suitable conditions must be fulfilled, and these conditions may differ with different micro-organisms. On the other hand, filterability does not mean that the micro-organism is necessarily "ultramicroscopic" during all the phases of its development. For some years the virus of yellow fever was thought to be ultramicroscopic because of the ease with which it would pass through an earthenware filter; but Noguchi [25] has lately established the spirochetal nature of the micro-organism.

Other biologic characters, moreover, place the etiologic agent of trench fever in close relationship with the group of filter-passing viruses. Mention has already been made of the manner in which the virus in infectious urine resists the concentration of salts resulting from desiccation. Byam and his co-workers have demonstrated that the virus in the excrement of lice retains its activity for at least one hundred and twenty days, even though it is exposed to the ordinary laboratory temperature and humidity, and to sunlight They [26] have also shown that it is not killed by several weeks' exposure to 50 per cent. glycerin. In their hands it resisted dry heat of 80.5 C. for twenty minutes, but was killed by exposure to 100 C. dry heat for a similar period. When moist heat was applied, it was killed by twenty minutes' exposure at 60 C. In our experiments the virus in infected louse excrement resisted 60 C. moist heat for one-half hour, but was killed after exposure to 70 C. moist heat for a similar period. The discrepancy can probably be explained by the fact that only one series of tests was carried out by each commission; no doubt heat resistance experiments would more nearly correspond, if several series were performed. The important lesson to be drawn from both experiments is that higher degrees of heat are necessary to disinfect the excreta than are required to free clothing from lice and viable eggs.

The low thermal death point of the virus demonstrates that the other resisting qualities of the micro-organism are not due to ordinary bacterial spores. The peculiar behavior of the trench fever virus in the presence of various physical and chemical agents practically rules out the possibility that it belongs to the spirochete group.

Aside from some unsubstantiated claims that a spirochete is the etiologic agent in trench fever, the most suggestive finding, from the morphologic point of view, is that of the so-called Rickettsia bodies. These bodies were first described by Ricketts [27] in the blood of patients suffering from Rocky Mountain spotted fever and in the bodies of the ticks that transmit this disease. A short time later similar bodies were observed by Ricketts and Wilder [28] in the study of typhus fever.

25. Noguchi, H.: J. Exper. M. **30**:13, 1919.
26. Personal communication of Dr. Byam to Dr. Harold Amoss.
27. Ricketts, H. T.: J. A. M. A. **52**:379, 1910.
28. Ricketts, H. T., and Wilder, R. M.: J. A. M. A. **54**:1373 (April 22) 1910.

These findings in spotted fever have been amply confirmed by Wolbach.[29] In typhus, in addition, Prowazek,[30] da Rocha-Lima,[31] and many other observers have shown that lice which have fed on patients with this disease pass large numbers of the bodies in their excreta, and also harbor many of them in the epithelial cells of the intestinal mucosa.

Morphologically, they are small bodies that vary in size from 0.3 to 0.5 by 1.5 microns. In shape, they present various outlines: cocci, diplococci and short bacilli. In the diplococcoid form the two bodies are often joined by a faintly staining substance so that dumbbell or figure 8 forms are seen. Observed under the dark field microscope, these forms have a tumbling motion, but possess no distinct motility of their own. They stain readily in films with either Giemsa or concentrated gentian violet; they are gram-negative, and not acid-fast. With Giemsa stain they take a red violet color of much the same shade as that of the nucleus of a polymorphonuclear leukocyte. Arkwright[32] states that he can distinguish the Rickettsia in lice that have fed on trench fever patients from those that have fed on typhus fever patients by the following characteristics: In trench fever fed lice the bodies are more purplish and smaller; in typhus fed lice they are larger and redder. In blood they are best demonstrated in thick drop preparations from which, after drying, the hemoglobin has been removed by distilled water or acid alcohol. Because of their small size and small numbers in the circulating blood, it is often necessary to make prolonged examination of blood films, in order to demonstrate them. They are much more easily found in the bodies of the insect vectors of these diseases. Attempts to cultivate the pathogenic forms on artificial media have resulted in failures; but both Nöller[33] and Jungmann[34] report that they have succeeded in cultivating on dextrose serum agar the Rickettsia bodies found in sheep ticks. The latter observer has shown that this species, the Rickettsia melophagi, is simply a parasite of the tick and does not produce any disease in the sheep harboring the insects. Films made from the cultures of Rickettsia melophagi show all of the forms that are seen in the excrement and the bodies of lice, and in the blood of patients.

Early in their studies various German observers described small micro-organisms that resembled the Rickettsia bodies of typhus fever in the blood of patients suffering from trench fever. This finding, combined with the supposed similarity in the mode of spread of the

29. Wolbach, S. B.: J. Med. Res. **34**:121, 1916.
30. Von Prowazek, S.: loc. cit.
31. da Rocha-Lima H.: München. med. Wchnschr. **64**: 1917.
32. Arkwright, J. A.: Proc. Roy. Soc. Med. **13**:23, 1919.
33. Nöller: Berl. klin. Wchnschr. **54**:346, 1917.
34. Jungmann, P.: (Monograph) Das Wolhysische Fieber, Berlin, 1919.

two diseases led Töpfer,[35] Jungmann and Kuczynski,[36] and da Rocha-Lima [37] to search for these bodies in the excrement and bodies of lice that had fed on patients with Wolhynian fever. Their demonstration of Rickettsia bodies in the intestinal mucosa and excrement of these lice was the chief support for their hypothesis that lice were the insect vectors of this disease. They reported that these bodies could not be found in lice until the lapse of at least five days after the insects had fed on a patient. This time corresponded so closely with the period between relapses in patients with spiky periodic fever that the German observers felt this fact furnished a further support to the hypothesis of the etiologic relationship of these bodies. Jungmann states that he was able to find them in the blood of patients with the spiky type of relapses only at the time of the relapses; on the other hand he found them in the blood of patients with continuous or typhoid type of pyrexia at any time during their fever. In spite of the attractiveness of this evidence as to the etiologic rôle of Rickettsia, it does not correspond with the findings of the British commission as to the infectivity of patients for lice. In the experiments of the last named observers, lice could be infected equally well by feeding on patients during the febrile and afebrile periods. Jungmann's observations, therefore, merely indicate a correspondence between the demonstrable presence of Rickettsia bodies in the blood and the occurrence of fever.

Jungmann and Kuczynski [38] claim that they were able to find these bipolar bodies in the blood of mice that had been inoculated with the blood of patients or with the excrement of lice that had fed on patients. Da Rocha-Lima,[39] on the contrary, was unable to infect mice, but reported that he produced the typical disease in seven out of forty-four guinea-pigs inoculated with blood, urine or lice from trench fever patients. He was, however, unable to pass the infection on to a second generation of the animals. In a series of experiments in which he allowed normal lice to bite seventy trench fever patients, 72 per cent. of the insects showed Richettsia bodies; on the other hand, 20 per cent. of the lice that had fed on patients who were supposed not to have had trench fever also showed the bodies. He explained these findings on the assumption that normal lice might be infected with a non-pathogenic type of micro-organism that he called Rickettsia pediculi. Probably a better explanation is that the patients were suffering from atypical trench fever or that some of them had a trench fever complicating the disease for which they were admitted to the hospital.

35. Töpfer, H.: München. med. Wchnschr. **63**:1495, 1916.
36. Jungmann, P., and Kuczynski, M. H.: Berl. klin. Wchnschr. **53**:323, 1916.
37. da Rocha-Lima, H.: Berl. klin. Wchnschr. **53**:567, 1916.
38. Jungmann, P., and Kuczynski, M. H.: loc. cit.
39. da Rocha-Lima, H.: Loc. cit.

Jungmann,[40] in contradistinction to da Rocha-Lima, states that he has never found Rickettsia bodies in lice that have not fed on either trench fever or typhus fever patients. Both of these observers claim that the interval between an infecting feed and the time that the Rickettsia bodies appear in the insects is about five days for trench fever and from nine to twelve days for typhus fever.

The British commission has confirmed with certain exceptions the observation of the German authors. Arkwright, Bacot and Duncan,[41] who carried out this portion of the work, had the advantage of working with a pedigreed stock of lice, as well as with experimentally produced cases of the disease from which to infect the insects. Finally, they were able to compare the appearance of the Rickettsia bodies with the infectivity of the lice for normal subjects. They [42] have lately reported that they were able to find Rickettsia bodies in all of 108 boxes of lice that had fed several times on sixty-four trench fever patients. In only one out of many lots of the insects fed only on normal persons, were forms found that suggest trench fever Rickettsia bodies. In their experiments, a volunteer inoculated with a single louse that contained the Rickettsia bodies contracted trench fever, while another subject inoculated with a single louse from the same box, but free from Rickettsia bodies, remained well. There was also a remarkable correspondence between the appearance of Rickettsia bodies in the excrement of lice after the infecting feed and the virus content of the same excrement when inoculated into susceptible volunteers. The interval between the infecting feed and the appearance of the bodies varied from five to twelve days, with an average of from seven to ten days. This time is longer than that reported by the Germans, but corresponds closely with the average incubation period in patients inoculated with the excreta of infected lice. Lice that were fed on patients with experimentally produced trench fever during the first day only did not subsequently show Rickettsia bodies; although lice fed later on the same patients did show them. In this connection it is of interest to note that the only lice that were usually not infectious in our original experiments were those that were fed on patients during the last two days of the incubation period and during the first two or three days of the fever. Blood from these same patients, however, contained virus, as demonstrated by intravenous inoculation. In all types of experiments, with one exception, therefore, there is a striking parallelism between the infectivity of the insect vectors and the appearance of Rickettsia bodies. This exception was reported by Arkwright

40. Jungmann, P.: (Monograph), loc. cit.
41. Arkwright, J. A., Bacot, A., and Duncan, F. M.: J. Hygiene **18**:76, 1919.
42. Arkwright, J. A.: Proc. Roy. Soc. Med. **13**:23, 1919.

and Byam [43] and is as follows: Two lots of lice were allowed to feed on a trench fever patient; one lot was kept at a temperature of from 27 to 30 C. and developed Rickettsia bodies; the other lot was kept at 17 C. and did not develop them. The excrement from both lots of lice proved to be infectious by inoculation into normal volunteers.

While it is difficult not to believe that there is a causal relationship between the virus of trench fever and the Rickettsia bodies, it will be difficult to establish definitely such a relationship until it is possible to obtain pure cultures of the bodies and with them to reproduce the disease. In this connection it must be recalled that the relation of Rickettsia bodies to other micro-organisms has not been established. They may be specific micro-organisms; they may be a granular stage through which some other micro-organism is passing; or, finally, they may be cell inclusions, the result of the action of some invisible virus on the cell protoplasm, and thus resemble the Guarnieri bodies in vaccinia, the Negri bodies of rabies, the molluscum bodies in molluscum contagiosum and the cell inclusions in trachoma.

In our efforts to determine the pathogenesis and histologic changes in this disease, we are handicapped by inadequate knowledge as to the nature of the virus and by a total lack of necropsies. The non-fatal character of the disease has made it impossible to examine thoroughly all of the tissues of the body for the site of attack of the virus. In addition, the failure to reproduce the typical disease in lower animals has forced us to resort entirely to the study of the various clinical manifestations in man, in order to arrive at some understanding of the nature of the infection.

In the two other well known diseases that are associated with the appearance of Rickettsia bodies in the insect vectors of the virus, it has been established that the chief structures showing definite histologic changes are the small blood vessels. Wolbach [44] has shown that the reaction in Rocky Mountain spotted fever is "an endangitis, characterized by endothelial cell proliferation, local necrosis of endothelium and smooth muscle, and thrombosis. Perivascular accumulations of large mononuclear cells are of common occurrence." The lesions are limited practically to the skin and genitalia. Fraenkel [45] has demonstrated that the essential lesion in typhus fever is the same; but in the latter disease the vessels of the viscera are also involved. Schminke [46] has compared the histologic changes in the exanthems of trench fever and of typhus, and shown that in the hyperemic and edematous corium of trench fever macule there is a perivascular lymphocytic infiltration mixed

43. Report of British Trench Fever Commission, loc. cit.
44. Wolbach, S. B.: J. Med. Res. **37**:499, 1918.
45. Fraenkel, E.: München. med. Wchnschr. **62**:805, 1915.
46. Schminke, A.: München. med. Wchnschr. **64**:961, 1917.

with some polymorphonuclear leukocytes. The endothelium and vessel wall necrosis and hyaline thrombosis, found in typhus fever lesions, was entirely absent. This probably explains why the trench fever exanthem is not petechial. In typhus fever the intensely toxic nature of the virus leads to an actual death of the cells and often of the patient; while in trench fever the less toxic virus does not lead to a destruction of either cell or host. A similar action on the body of the insect vector of the two diseases has been found. Both the English and German observers have noted that lice that have bitten trench fever patients live their normal number of weeks; on the other hand, Jungmann and da Rocha-Lima call attention to the fact that the life of lice is shortened by feeding on typhus fever subjects.

Clinically, the main tissues that seem to be involved in trench fever, aside from the skin, are the hematopoietic organs and the nervous system. The polymorphonucleosis during the febrile paroxysms, followed by an increase in the mononuclear elements of the blood, and the peculiar enlargement of the spleen all indicate that the virus has a marked effect upon the blood forming and blood destroying organs. There has been much discussion as to the cause of the peculiar pain and tenderness in patients with this disease. These symptoms are not accompanied by other signs of local inflammation of the periosteum, muscles or tendons, such as swelling, redness or edema. The description of the pains given by many patients resembles the pains that occur in the early stages of tabes dorsalis. Byam,[47] Carmalt Jones,[48] and others, have called attention to the peculiar distribution of cutaneous hyperesthesia in the areas supplied by the eighth cervical, first and seventh dorsal, and all the lumbar segments of the cord, during the active stages of the disease. Sundell [49] has observed that later there is a distinct blunting of the cutaneous sensibility over similar areas. These sensory disturbances, coupled with the increase in tendon, cutaneous and pilomotor reflexes, all point to some abnormal condition of the sensory tracts, probably in the region of the dorsal roots. The condition of "disordered action of the heart," a late complication in certain patients, can best be explained on the basis of a disturbance of the autonomic nervous control of the cardiac action. In patients suffering from this peculiar group of symptoms or from neurasthenia following an attack of acute trench fever, there is usually evidence that the disease is still active in a chronic form.

The many forms of fever that have resulted from the artificial inoculation of different individuals with the same strain of virus have

47. Byam, W., Dimond, L., Sorapure, V. E., and Wilson, R. M.: J. R.A.M.C. **29**:560, 1917

48. Carmalt Jones, D. W.: Lancet **2**:443, 1918.

49. Sundell, C. E.: Lancet **2**:538, 1918.

demonstrated that the various clinical types of the disease are not due to different varieties of the micro-organism, as is the case in malaria. The spiky type of paroxysm, requiring but a single day for its completion, can best be explained on the assumption that the virus requires a certain time for its complete development in the tissues of the patient; when that period is complete, the micro-organisms or a toxin that they develop as a result of their activity, flood the patient, and give rise to the explosive picture. If, on the other hand, the micro-organisms are of different age, either as a result of multiple inoculation, or because of a mixture of virus of different ages, they will attain their maximum growth in the patient's body at different times, and produce a septicemic or typhoid type of fever.

Recovery from the disease is evidently due to the development of an immunity on the part of the patient. The time required for the development of this immunity varies within wide limits. No doubt some individuals possess a complete immunity to the infection. In others there is a partial immunity, so that the introduction of the virus into their bodies results in abortive or larval types of the disease. In the majority of patients, complete immunity develops only after repeated flooding of the body with the virus; and, on the average, requires from three to six weeks for its production. Even then it may be only partial—sufficient to hold in abeyance all symptoms until the patient is subjected to some general depressing influence, when a relapse occurs. Among our volunteers we had a number of examples of such relapses after prolonged periods of absence of fever and symptoms; and lately I have seen in a physician a relapse that occurred twenty-six months after the original attack in Flanders. Such prolonged periods of freedom from symptoms with subsequent relapses remind one of similar conditions in malaria and syphilis.

In still another group of patients months or years are required for complete immunity to develop. This group comprises from 5 to 10 per cent. of all of the patients afflicted with the disease. In them the manifestations assume a subacute or chronic form; the patients are never entirely free from symptoms; occasional low grade fever is found. The condition is variously described by the terms myalgia, neuralgia, neurasthenia, disordered action of the heart, or trench fever cachexia. Byam and his associates have shown that at least some of these sufferers are carrying the virus in their blood as late as from three to four hundred days after onset of fever.

The immunity that develops with recovery is of relatively short duration. The British commission showed that reinfection was possible on the one hundred and thirty-second and one hundred and ninety-

eighth days after the onset; Werner [50] reinfected himself six months after his first attack. On the other hand, the British commission found it impossible to reinfect some patients at periods varying from sixty-two to 182 days after the onset. Irregularity in the duration of immunity seems to be as much a feature of the disease as irregularity in the time of development of immunity and as irregularity in most of the symptoms.

The practical application of all the knowledge that has been gained by much effort is that eradication of the louse in infected bodies of men is followed by a cessation of the disease. The discussion that has arisen as to whether the chief mode of human infection is through the bite of the insects or results from introduction of louse excretion into excoriated skin is more academic than practical. The method of inoculation by applying louse excrement to scarified skin has resulted in much useful knowledge concerning both human and insect carriers of the disease. It has been the experience of many observers that the wholesale application of measures against louse infestation has been followed by a diminution in the incidence of the disease. This was strikingly brought out in the experience in the Third Army of the American Expeditionary Forces.[51] The experience in the Presbyterian Base Hospital Unit [52] of this city showed that infected and infested clothing and equipment may be handled with impunity, provided the people handling such material are protected against lice. The chance for contracting infection from patients was as great, or greater, after May, 1918, as before, but the simple institution of effective measures against the possibility of becoming infested with lice from the patients, resulted in practical freedom from new infections among nurses and orderlies.

SUMMARY

During the recent war a disease, hitherto unrecognized as a clinical entity, was widespread throughout the armies on both the eastern and western fronts. Although the manifold forms of the affection make accurate statistics impossible, it is estimated that between 800,000 and 1,000,000 cases must have occurred. Before the influenza epidemic it was the most frequent single disease in several of the armies. While not fatal, it usually resulted in disability for from ten to twelve weeks, and in 10 per cent. of the cases was the cause of invalidism for many months. In such instances the infection is active in a chronic form. The many clinical forms of the disease are apparently not due to the

50. Werner, H., and Benzler, J.: München. med. Wchnschr. **64**:695, 1917.
51. Swift, Homer F.: J. A. M. A. **73**:807 (Sept. 13) 1919.
52. Prevention of Trench Fever Among Hospital Personnel, Mil. Surgeon **44**:370, 1919.

action of different types of micro-organism, but to single or multiple infections with a single type of organism. The intensity and duration of an attack seem to depend on the relation between the infectivity of the virus and the immunity in the patient.

It has been demonstrated that the disease is not a modified form of enteric or typhus fever, but that it is due to a specific infectious agent. This etiologic agent behaves in the presence of various physical and chemical environments in a manner similar to that of many of the filter passing micro-organisms. Under suitable conditions the virus of trench fever will pass through the pores of a filter that are small enough to hold back ordinary bacteria. The virus is found occasionally in the sputum of patients, often in the urine, and always in the blood at some stage. It is also found in the excrement and bodies of practically all lice that have fed several times on trench fever patients, after an interval of from five to ten days following the infecting feed. After a louse has started to excrete active virus, it continues to do so for the remainder of its life. The virus is not transmitted to the larvae of lice through the eggs. The interval elapsing between the time of the infecting feed and the first excretion of virus by lice closely corresponds with the length of the incubation period in men inoculated with a maximum dose of virus. There is a remarkable correspondence in the infectivity of louse excrement and the time of appearance of Rickettsia bodies; these bodies are also demonstrable, with difficulty, in the blood of patients during the period of pyrexia. The etiologic rôle of Rickettsia bodies, however, as well as the relation of these bodies to micro-organisms in general, remains to be established.

While men may be infected by the simple bites of infected lice, they are more surely infected by applying the excrement of such lice to scarified skin; infected lice, living under normal conditions, transmit the disease to the majority, if not to all, men harboring them. As a direct corollary, the eradication of lice is followed by an eradication of the disease.

A STUDY OF PHOSPHATE RETENTION FROM THE STANDPOINT OF BLOOD ANALYSIS *

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During the past five years our knowledge regarding the content of inorganic phosphates in blood has been advanced by the work of several investigators: Taylor and Miller,[1] Greenwald,[2] Marriott and Howland,[3] Feigl [4] and Bloor [5] have published the results of determinations of the inorganic phosphate content of plasma. Each investigator has worked by a different method, but, with the exception of the results obtained by Taylor and Miller, who state that the inorganic phosphate content of plasma is so small as to be negligible, the average values reported by the other workers are within the same general range.

In certain cases of nephritis, Greenwald and Marriott and Howland have reported the presence of greatly increased amounts of inorganic phosphates in plasma, the importance of which finding, in connection with both the theoretical and practical aspects of nephritic acidosis, has been pointed out by the latter workers.

More than a year ago, on the publication of Bloor's exceedingly simple method for the determination of inorganic phosphates in plasma, we began the accumulation of data on the subject with the immediate object of finding out whether a knowledge of the inorganic phosphate content of plasma might be of value in the prognosis or diagnosis of renal disease. As a preliminary to our work on nephritic conditions, it seemed necessary, in view of the relatively small number of figures to be found in the literature, to make determinations of inorganic phosphates in the plasma of patients suffering from conditions other than renal or cardiorenal. A statement of the values obtained with this class of material is contained in Table 1. In many cases the nonprotein nitrogen was also determined, and where available these figures have been included in the tabulated results.

From the results presented in Table 1 it would seem that the inorganic phosphates in serum in various pathologic conditions other than nephritis or cardiorenal disease, may vary from 1.2 to 3.1 mg. of phosphorus per hundred c.c. of plasma. Bloor's results on twenty-seven normal persons give slightly higher values, i. e., minimum 1.8 and maximum 4.3 mg.

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* From the Chemical Laboratory of the Massachusetts General Hospital.

1. Taylor, A. E., and Miller, C. W.: J. Biol. Chem. **28**:205, 1914.
2. Greenwald, I.: J. Biol. Chem. **29**:21, 1915.
3. Marriott, W. McK., and Howland, J.: Arch. Int. Med. **18**:708, 1916.
4. Feigl, J.: Chem. Abstr. **11**:3316, 1917; **12**:809, 1918.
5. Bloor, W. R.: J. Biol. Chem. **36**:49, 1918.

TABLE 1.—Inorganic Phosphates in Blood

Case No.	Mg. per 100 C.c. Plasma		Diagnosis
	Inorganic Phosphates as P.	Non-protein Nitrogen	
20	1.2	40	Anemia (secondary)
10	2.0	..	Arthritis (chronic)
30	2.0	38	Arthritis (chronic)
40	3.1	52	Arthritis (chronic)
88	2.1	40	Arthritis (chronic)
70	1.8	44	Arthritis (acute)
73	2.7	37	Arthritis (acute)
150	2.2	44	Arthritis (acute)
164	2.4	31	Arthritis (acute)
177	2.3	41	Arthritis (acute)
180	2.5	34	Arthritis (acute)
194	2.6	30	Arthritis (acute)
196	2.4	36	Arthritis (acute)
211	2.3	40	Arthritis (acute)
232	2.0	34	Arthritis (acute)
258	3.2	37	Arthritis (acute)
25	1.8	..	Diabetes
32	1.4	..	Diabetes
34	1.9	..	Diabetes
38	2.0	..	Diabetes
43	1.6	..	Diabetes
44	2.6	32	Diabetes
54	1.8	42	Diabetes
62	1.7	30	Diabetes
74	2.6	35	Diabetes
144	2.6	..	Diabetes
208	2.8	42	Diabetes
24	1.8	30	Influenza
99	2.1	38	Influenza
42	1.6	46	Influenza
7	1.8	32	Gastric ulcer
5	1.8	42	Gastric ulcer
16	2.6	32	Gastric ulcer
143	2.3	38	Gastric ulcer
19	1.6	32	Gastric cancer
35	2.6	50	Gastric cancer
97	2.1	30	Gastric carcinoma
189	2.4	47	Gastric carcinoma
190	2.7	43	Gastric carcinoma
9	1.6	..	Jaundice (catarrhal)
29	3.1	34	Jaundice (catarrhal)
79	2.2	40	Jaundice (catarrhal)
51	2.6	38	Hyperthyroidism
63	1.8	40	Hyperthyroidism
163	3.0	33	Hyperthyroidism
56	3.1	48	Syphilis (cerebrospinal)
84	2.2	35	Syphilis (cerebrospinal)
195	1.7	40	Syphilis (cerebrospinal)
220	2.7	44	Syphilis (cerebrospinal)
224	2.1	45	Syphilis (cerebrospinal)
231	3.2	33	Syphilis (cerebrospinal)
233	2.3	37	Syphilis (cerebrospinal)
234	2.8	37	Syphilis (cerebrospinal)
139	3.1	40	Pneumonia (bronchial)
144	1.8	32	Pneumonia (lobar)
145	2.3	42	Pneumonia (lobar)
157	1.5	44	Pneumonia (lobar)
206	2.9	40	Pneumonia (lobar)
138	1.3	52	Pulmonary tuberculosis
148	2.3	30	Pulmonary tuberculosis
57	1.8	..	Psoriasis
86	3.0	..	Psoriasis
141	2.0	31	Psoriasis
59	1.8	..	Chronic eczema
101	1.3	36	Chronic eczema
115	1.8	..	Chronic eczema
134	2.6	40	Chronic eczema
238	3.1	38	Chronic eczema
179	2.7	42	Chronic eczema
239	2.8	35	Herpes zoster
6	1.8	37	Torticollis
45	2.9	32	Normal pregnancy
69	1.8	68	Gangrene of leg
71	1.8	44	Gangrene of leg
200	2.0	45	Osteomalacia
28	2.1	44	Cholelithiasis
49	2.8	60	Pituitary tumor
140	2.6	57	Acute endocarditis
127	2.1	47	Lead poisoning (chronic)
233	2.2	35	Valvular disease (chronic)

The results obtained on sixty-eight cases of renal and cardiorenal disease have been collected in Table 2. These cases have been arranged according to the level of plasma phosphates, and without regard to the anatomic nature of the lesions.

From the figures collected in Table 2 it is apparent that many nephritic and cardiorenal cases sufficiently ill to enter a hospital show no retention of inorganic phosphate in their plasma. Out of the sixty-eight cases included in this tabulation, twenty four (about 35 per cent.) showed an inorganic phosphate content of plasma equal to or below the maximum obtained in the miscellaneous material examined, the results on which are shown in Table 1. In view of the fact that our cases were not selected, and that we simply made use of all material available during the period in which this study was in progress, it is, perhaps, not unjustifiable to conclude that at least 60 per cent. of the ordinary cases of nephritis with or without cardiac involvement, show a retention of inorganic phosphate in the plasma.

In Table 3 we have grouped together eleven cases of nephritis and allied conditions on which we were able to make several blood examinations during the course of the patient's stay in the hospital and to thus learn something of the changes in the level of plasma phosphate which may occur when the clinical condition of the patient is improving, and when the symptoms are increasing in severity.

An inspection of the results presented in Table 3 shows that of the eleven cases included in the group, nine patients died, while two were discharged relieved.

In all fatal cases the "premortal" rise in plasma phosphate is most striking. It will also be noted that in the two nonfatal cases the plasma phosphate, while distinctly above our maximum "normal" figure, did not, at any time, rise to an alarming height. The lack of concordance between the increased content of inorganic phosphate and the nonprotein nitrogen has been noted by Greenwald, and by Marriott and Howland; our results offer numerous confirmations of the findings of these investigators.

We wish also to call attention to the lack of any definite relation between the plasma phosphate and the alkaline reserve, a finding not without interest when viewed from the standpoint of modern theories of acid base equilibrium.

In this connection it may be of interest to state that we have had several opportunities of confirming the findings of Marriott and Howland regarding the entire lack of effect produced by sodium bicarbonate medication on the level of plasma phosphate.

In conclusion, it may be said that, as far as one can judge from the limited data available, the determination of the inorganic phosphate of the plasma gives promise of being of value in connection

TABLE 2.—Inorganic Phosphates in Plasma in Nephritis and Cardiorenal Disease

Case No.	Mg. per 100 C.c. Plasma		Alkal. Reserve Con. Value per Cent.	
	Inorganic Phosphate as P.	Non-protein Nitrogen		
14	1.9	33	..	Chronic nephritis, hypertension
147	1.9	32	..	Acute nephritis
110	1.9	86	..	Chronic nephritis, hypertension
149	2.0	39	46	Arteriosclerosis, chronic nephritis
151	2.0	40	..	Cardiorenal disease
21	2.1	..	..	Arteriosclerosis; senile heart; chronic nephritis
16	2.2	32	..	Chronic nephritis, hypertension
53	2.2	38	..	Acute nephritis
136	2.2	34	..	Cardiorenal disease
158	2.4	28	..	Subacute glomerulonephritis
256	2.4	30	51	Senile arteriosclerosis; chronic nephritis
192	2.5	46	42	Arteriosclerosis; chronic myocarditis; chronic nephritis
271	2.5	38	60	Arteriosclerosis, chronic nephritis
156	2.5	50	66	Arteriosclerosis, hypertension; chronic nephritis; diabetes
15	2.6	40	..	Arteriosclerosis; cardiorenal disease
29	2.6	60	..	Chronic interstitial nephritis
47	2.6	40	..	Mercury poisoning, acute
100	2.6	75	..	Chronic nephritis
108	2.6	47	..	Mercury poisoning, acute
117	2.7	66	..	Chronic nephritis
77	3.1	84	..	Chronic glomerulonephritis
283	3.1	66	..	Chronic nephritis, hypertension; albuminuric retinitis
105	3.1	62	..	Chronic nephritis
196	3.1	48	42	Cardiorenal disease
88	3.2	64	..	Chronic nephritis
90	3.2	41	..	Chronic nephritis
37	3.4	40	..	Mercury poisoning, acute
96	3.4	40	..	Arteriosclerosis, chronic nephritis
105	3.4	36	..	Chronic nephritis
107	3.4	37	..	Chronic nephritis
267	3.5	96	33.6	Chronic nephritis, uremia
169	3.6	36	..	Chronic nephritis, uremia
223	3.6	44	57	Cardiorenal disease
168	3.7	40	46.3	Syphilis, chronic nephritis
228	3.7	45	..	Cardiorenal disease
102	3.8	76	32.8	Subacute nephritis, bronchopneumonia, uremia
104	3.8	34	..	Chronic nephritis
235	3.9	80	30	Chronic nephritis, hypertension
174	4.0	40	..	Chronic nephritis, hypertension
72	4.0	..	..	Aortic and mitral insufficiency, chronic nephritis
109	4.1	85	..	Chronic nephritis
206	4.4	55	32	Chronic nephritis
219	4.6	56	54	
248	4.2	55	59	Myocardial weakness, hypertension, chronic nephritis
220	4.3	32	56	Chronic nephritis
204	4.4	40	49	Cardiorenal disease, diabetes
218	4.4	40	42	
199	4.4	34	..	Chronic nephritis, amyloid kidney, uremia
209	4.4	190	..	Chronic glomerular nephritis, uremia
160	5.3	143	30	Cardiorenal disease, uremia
222	5.3	56	52	Chronic nephritis
8	5.3	55	..	Chronic nephritis
257	5.3	47	48	Chronic nephritis
226	5.3	50	46	Cardiorenal disease
161	5.6	161	30	Subacute glomerular nephritis, uremia
252	16.0	164	46	Chronic glomerular nephritis, uremia
207	6.1	47	..	Chronic nephritis
205	6.0	50	50	Chronic nephritis
209	6.0	190	37	Chronic glomerular nephritis, uremia
270	6.0	164	33	Chronic nephritis
67	6.2	36	..	Hypertension, arteriosclerosis, chronic nephritis
247	6.2	74	45	Hypertension, chronic nephritis
182	7.8	54	49.6	Chronic nephritis, hypertension
275	8.4	118	..	Chronic nephritis, hypertension
106	8.5	63	..	Chronic nephritis
154	10.3	180	..	Pyelonephritis
289	12.4	237	21	Chronic nephritis, hypertension, arteriosclerosis, uremia
290	12.4	298		
284	18.0	205	..	Chronic myocarditis, chronic nephritis, uremia
236	14.8	192	..	Chronic nephritis, hypertension, uremia
237	22.3	222	46	
205	28.1	50	51	Myocardial insufficiency, chronic nephritis

with progress in renal and cardiorenal disease. We have noted cases in which the nonprotein nitrogen was high, the phenolsulphonephthalein output extremely low, and the plasma phosphate but slightly increased. These patients, whatever may have been their later fate, invariably left the hospital relieved of their more serious subjective symptoms. An example of this type is Mr. M. (Table 3).

On the other hand, many, although we are not prepared to say all, fatal cases show a rapidly rising plasma phosphate.

TABLE 3.—Changes in Level of Plasma Phosphate in Nephritis and Allied Conditions

Date, 1919	Mg. per 100 C.c. Plasma		Alkali Reserve Con. Value per Cent.	
	Inorganic Phosphate as P.	Non-protein Nitrogen		
Jan. 18	6.0	72	....	Mr. T. Chronic glomerular nephritis, uremia. Died
Jan. 20	6.0	82		
Jan. 30	10.3	122		
Jan. 31	15.6	200	43.0	
Jan. 14	15.5	100	....	Mr. B. Hypertension, arteriosclerosis, cardiorenal disease, uremia. Died one hour after last sample of blood was taken
Jan. 16	43.7	113		
Jan. 18	43.7	180		
Jan. 28	2.0	56	....	Mr. S. Aortitis, syphilis, cardiorenal disease. Died
Jan. 30	2.0	40		
Feb. 1	2.0	40		
Feb. 5	9.1	62		
Feb. 13	3.4	68	....	Mrs. P. Mercuric chlorid poisoning. Died three days after last sample of blood was taken
Feb. 15	3.9	100		
Feb. 16	4.5	125		
Feb 18	9.2	140		
Feb. 13	3.5	62	....	Mr. S. Aortic stenosis and regurgitation, chronic interstitial nephritis. Died
Feb. 19	3.5	65		
Feb. 27	6.5	90		
Mar. 10	2.8	105	....	Mr. B. Chronic glomerular nephritis, uremia. Died
Mar. 15	9.4	167	27.0	
Mar. 19	15.0	200	26.2	
Mar. 15	6.5	153	....	Mrs. T. Chronic glomerular nephritis, uremia. Died
Mar. 19	7.5	250	28.1	
Mar. 20	8.1	278	27.0	
April 21	4.5	143	24.2	Mr. M. O. Chronic interstitial nephritis uremia. Died. On April 26 sodium bicarbonate was administered intravenously
April 25	10.0	152	24.2	
April 26	9.8	165	15.4	
April 26	9.8	165	26.0	
April 27	10.4	163	20.1	
July 2	3.6	69	54.0	Mr. D. Chronic glomerular nephritis. Died
July 10	9.4	103		
July 15	11.6	140		
July 18	17.7	160	24.1	
July 22	18.9	208	22.4	
Sept. 3	4.3	166	....	Mr. B. Jaundice (cause unknown), emphysema, nephritis. Discharged relieved
Sept. 6	4.0	75		
Sept. 16	2.8	36		
Sept. 26	2.4	39		
Mar. 10	2.3	72	....	Mr. M. Chronic myocarditis, mitral regurgitation and stenosis, cardiac decompensation, chronic nephritis. Discharged relieved
Mar. 17	3.3	125		
Mar. 19	3.5	142		
Mar. 20	3.0	105	43.0	
Mar. 23	2.1	66	46.6	
Mar. 24	1.6	49	48.6	
Mar. 27	1.6	113		

SUMMARY

As a result of our study of the inorganic phosphate content of plasma, it has been shown that about 65 per cent. of the nephritic and cardiorenal cases, taken at random from the material found in the hospital wards, gave unmistakable evidence of phosphate retention. Fatal cases showed a rapid and progressive increase in plasma phosphate, increases of more than ten times the maximum normal value having been noted; the nonfatal cases, even though the patient was seriously ill, presented a relatively slight increase. The results obtained suggest the possible prognostic importance of the determination of inorganic phosphates in the plasma of persons suffering from nephritis and allied disorders.

A NEW ELECTRODE FOR USE IN CLINICAL ELECTROCARDIOGRAPHY *

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It has become sufficiently clear that if in taking human electrocardiograms there is overshooting of the string, a high resistance has probably developed at the site at which the electrodes have been applied to an individual. Overshooting is, of course, undesirable because it deforms the curve. A high metallic resistance interposed in the circuit does not cause this defect. The deformity is seen either when an added resistance is introduced into the circuit or when a current passes. In the latter instance the overshooting is the greater, the greater the voltage. It has likewise become sufficiently clear, that if the resistance in the string-patient circuit is reduced below 2,000 ohms, the overshooting does not take place. It makes no difference so far as electrocardiography is concerned what is the reason of the overshooting—whether due to polarization at the electrodes or to a capacity effect in the cutaneous tissues as Pardee [1] has suggested.

TABLE 1.—RESISTANCES OBTAINED WITH VARIOUS ELECTRODES

Order of Taking Electrocardiograms	Type of Electrode	Figure	Resistance Before Salt Rub			Order of Taking Electrocardiograms	Figure	Resistance After Salt Rub		
			Lead 1 Ohms	Lead 2 Ohms	Lead 3 Ohms			Lead 1 Ohms	Lead 2 Ohms	Lead 3 Ohms
1	Plate	..	2,200	4,500	4,000	6	6	2,200	2,200	1,400
2	Lead	3	2,000	2,500	2,200	5	4	1,000	1,000	1,000
3	Immersion	..	4,000	3,000	3,400	4	5	2,500	2,500	2,000
7	German silver	..	.....	.....	.....	..	7	1,400	1,400	1,400

Provided, then, that the resistance is low, the form of electrode used is probably a matter of indifference. The electrode of choice is the one easiest to apply, easiest to keep in order, easiest and cheapest to make. At the Hospital of the Rockefeller Institute the electrode in use is made of a strip of lead foil [2] 7.5 cm. wide by 22.0 cm. long, and

* From the Hospital of the Rockefeller Institute.

* The occasion for making this study was the construction of the electrodes described in the text. The credit for devising them is due to Robert Neubuck, technician in this department. The satisfaction which their use has given has prompted the controlling tests which are now reported.

1. Pardee, H. E. B.: An error in the electrocardiogram arising in the application of the electrode, Arch. Int. Med. **20**:161 (Aug.) 1917.

2. The lead foil is an alloy of lead and tin known as roentgen-ray protection foil. The gage used is B. & S. 31. It is supplied in rolls 12 inches wide. The rubber strip is cut from rubber matting 1/16 inch thick, 3 feet wide, which may be obtained from the New York Belting and Packing Company.

of a strip of rubber sheet 9.0 cm. wide by 30.0 cm. long. The two strips are fastened together about 8.0 cm. from one end by a brass screw, brass binding post and washers, in the manner shown in Figures 1 and 2. No soldering is necessary. The manner of fastening permits the repeated use of the remaining portion of the lead foil if a break takes place at the binding post. This accident after prolonged use is of course unavoidable.

In order to test and to establish the usefulness of these electrodes, electrocardiograms made with them (Figs. 3 and 4) were compared with others taken with immersion-non-polarizable electrodes (Fig. 5), with the so-called plate electrodes (Fig. 6), and with German silver

TABLE 2.—MEASUREMENTS OF THE —

Electrodes	Figure		P_1*	P_2	P_3	Q_1	Q_2	Q_3
			Mm.	Mm.	Mm.	Mm.	Mm.	Mm.
Lead	3	Range	1.0-1.1	1.0	0.2 - 0.4	2.75-3.1	—	—
Lead	4		0.9-0.1	1.05-1.15	0.50- 0.62	2.4 -2.9	—	—
Immersion	5		1.00	1.0	0.43- 0.6	2.4 -2.65	—	—
Plate	6		1.0 - 1.5	1.2-1.5	0.6 - 0.8	2.5 -3.0	—	—
German silver	7		0.75- 1.2	0.9-1.0	0.8 - 0.1	2.55-2.8	—	—
Lead	3	Observed average	1.05	1.0	0.33	2.85	—	—
Lead	4		0.95	1.09	0.57	2.68	—	—
Immersion	5		1.0	1.0	0.50	2.53	—	—
Plate	6		1.07	1.31	0.71	2.72	—	—
German silver	7		0.91	0.97	0.92	2.69	—	—
			Mm. %	Mm. %	Mm. %			
Lead	3	Observed standard deflection†	21.51 = 107.5	21.5 = 107.5	20.13 = 100.65	Same as P_1	Same as P_2	Same as P_3
Lead	4		20.3 = 101.5	21.01 = 105.05	21.3 = 106.5			
Immersion	5		20.53 = 102.65	20.59 = 102.95	20.57 = 102.85			
Plate	6		21.08 = 105.4	21.05 = 105.25	20.43 = 102.15			
German silver	7		20.16 = 100.8	21.08 = 105.4	21.28 = 106.4			
Lead	3	Corrected average	0.96	0.93	0.33	2.85	—	—
Lead	4		0.94	1.04	0.54	2.64	—	—
Immersion	5		0.97	0.97	0.49	2.47	—	—
Plate	6		1.02	1.25	0.70	2.58	—	—
German silver	7		0.90	0.98	0.87	2.67	—	—

* P_1 = P wave in Lead 1; P_2 = P wave in Lead 2, etc.
† The standard deflection when 20 ohms are thrown into string circuit is 20.0 mm. If 20.0 mm. equals 100 per cent., a deviation from this height of the observed deflection may be expressed in per cent. This figure is used for correcting the observed average to 100 per cent.

electrodes (Fig. 7). The plate electrodes were fashioned after the manner of those introduced by the Cambridge Instrument Company; those of German silver after the pattern described by Williams;[3] the nonpolarizable ones in the manner which is usual when the extremities are immersed. The electrocardiograms were taken of a single individual at a single sitting in the order shown in Table 1.

After exposures noted in the first column were made (Table 1), the arms and leg of the patient were rubbed gently with warm saturated salt solution; then those recorded in the second column were taken. Taking all the electrocardiograms occupied about one hour. Slight

3. James, W. B., and Williams, H. B.: The electrocardiogram in clinical medicine, Am. J. M. Sc. **140**:408, 1910.

overshooting is seen in Figure 3 (the lead electrode before rubbing), and in Figure 5 (the immersion nonpolarizable electrode). On the whole, the resistance was highest when these two were taken. In comparing them, however, it is not the object to show the relation of resistance to the degree of overshooting, but to show the fact that the electrocardiograms, irrespective of the form of the electrodes, are closely comparable at resistances of this magnitude.[4]

That the curves are comparable is shown (Table 2) by the fact that the height of a wave in one lead, the R wave for instance in Lead 1, differs little from the other R waves in the same lead, that is to say,

—Waves in Figures 3 to 7

R_1	R_2	R_3	S_1	S_2	S_3	T_1	T_2	T_3
Mm.	Mm.	Mm.	Mm.	Mm.	Mm.	Mm.	Mm.	Mm.
18.4-19.0	9.5 -10.1	4.25-4.7	—	2.7-3.0	15.1 -15.9	1.95-2.1	1.9 -2.36	—0.65 - —0.7
17.4-18.26	8.7 - 9.13	4.25-4.5	—	1.5-2.0	13.8 -15.0	2.0 -2.3	1.85-2.15	—0.5 - —0.8
15.8-17.0	8.6 - 8.95	4.1 -4.85	—	2.2-2.65	12.7 -14.65	2.1 -2.2	1.75-2.1	—0.4 - —0.67
18.2-19.75	8.42- 8.85	3.85-4.25	—	1.2-2.0	13.75-15.4	2.5 -2.7	1.85-2.35	—0.6 - —0.87
17.7-19.2	8.55- 8.9	3.95-4.2	—	1.0-1.17	13.82-15.27	2.25-2.72	2.1 -2.35	—0.35 - —0.45
18.7	9.90	4.46	—	2.87	15.55	2.02	2.08	—0.68
17.68	8.97	4.44	—	1.67	14.64	2.18	2.00	—0.60
16.45	8.77	4.44	—	2.42	13.73	2.12	1.98	—0.50
19.04	8.70	4.08	—	1.61	14.88	2.56	2.08	—0.72
18.75	8.76	4.11	—	1.06	14.66	2.51	2.22	—0.38
Same as P_1	Same as P_2	Same as P_3	Same as P_1	Same as P_2	Same as P_3	Same as P_1	Same as P_2	Same as P_3
17.39	9.21	4.43	—	2.67	15.45	1.88	1.94	—0.68
17.43	8.86	4.17	—	1.89	13.75	2.15	1.90	—0.56
16.08	8.52	4.32	—	2.37	12.36	2.07	1.93	—0.49
18.06	8.27	3.95	—	1.53	14.57	2.43	1.94	—0.71
18.59	8.32	3.87	—	1.01	13.78	2.49	2.11	—0.36

the range of variation is small (Table 3). It is also shown (Table 2) that if a wave is small when taken with one electrode (for instance, P_1[5] taken with the German silver electrode), it does not follow that another wave (e. g. R_1) taken with the same electrode is also small, when compared with like waves (R_1) taken with other electrodes. (R_1 taken with the German silver electrode for instance is the tallest of the R waves in Lead 1). That in the same lead, the height of a given wave (R_1 for instance) varies is due probably to the influence of respiration.

4. A comparison of curves taken at higher resistances is not essential in this study, for it is admitted that overshooting occurs at higher resistances, and likewise that the amount of overshooting differs with different metals. The lead electrodes are perhaps more satisfactory in this respect than other similar ones.

TABLE 3.—Limits of Ranges of the Corrected Averages

Wave	Lead	Limits	Range
P	1	0.90 and 1.02 mm.	0.12 mm.
P	2	0.93 to 1.25	0.32
P	3	0.33 to 0.87	0.54
Q	1	2.47 to 2.67	0.20
R	1	16.03 to 18.59	2.56
R	2	8.27 to 9.21	0.94
R	3	3.87 to 4.43	0.56
S	2	1.01 to 2.67	1.66
S	3	13.36 to 15.45	2.09
T	1	1.88 to 2.49	0.61
T	2	1.90 to 2.11	0.21
T	3	0.36 to 0.71	0.35

In view of the practical identity of the heights of the waves all the electrodes described may be used interchangeably. The electrodes used by us are recommended because their construction is at least as simple as any other, and of the electrodes which are fastened to the limb they are smaller and easier to apply. They are by far the most comfortable, and can therefore be kept in place during prolonged observations. They answer the requirement that with them a low resistance can be attained, and a low resistance is the most important criterion in establishing the usefulness of electrodes. It is by no means uncommon to obtain resistances as low as 500 ohms.

Fig. 1.—Electrode in use at Hospital of Rockefeller Institute. The lead foil, rubber sheet and binding post are shown separately.

SUMMARY

With all electrodes, electrocardiograms are deformed by overshooting of the string, when the resistance developed at the site of the application is high.

Electrodes of simple construction are described. Electrocardiograms taken with them are identical with those taken by other electrodes, when the resistances are below 2,000 and comparable.

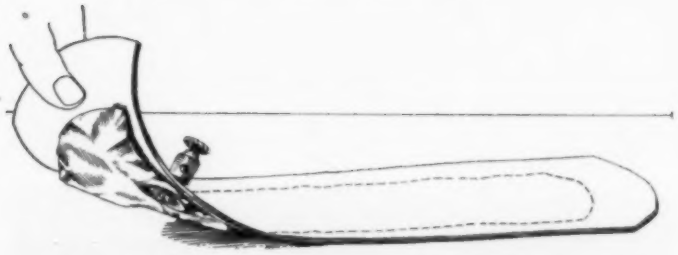

Fig. 2.—The electrode as assembled and ready for use.

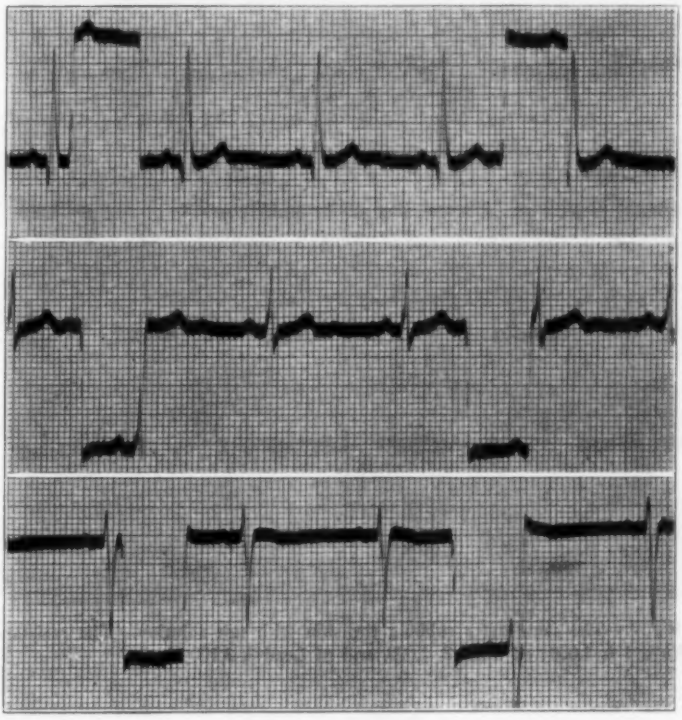

Fig. 3.—In all the electrocardiograms divisions of the ordinates equal 0.1 millivolt; divisions of the abscissae equal 0.04 second. The three usual leads are arranged from above downward. In each figure there are two deflections of about 20.0 mm. and of about 0.4 to 0.5 second duration. These are deflections caused by throwing 20 ohms into the circuit. This electrocardiogram was taken with lead electrodes before rubbing the skin with salt solution. The resistance of Lead 1 is 2,000; of Lead 2, 2,300; of Lead 3, 2,300 ohms.

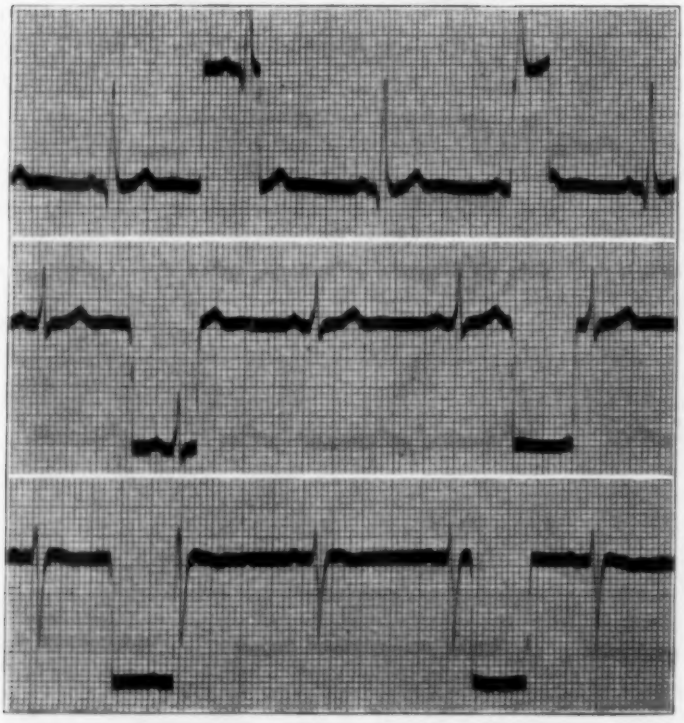

Fig. 4.—The same after rubbing the skin with salt solution. The resistance of Lead 1 is 1,000; of Lead 2, 1,000, and of Lead 3, 3,100 ohms.

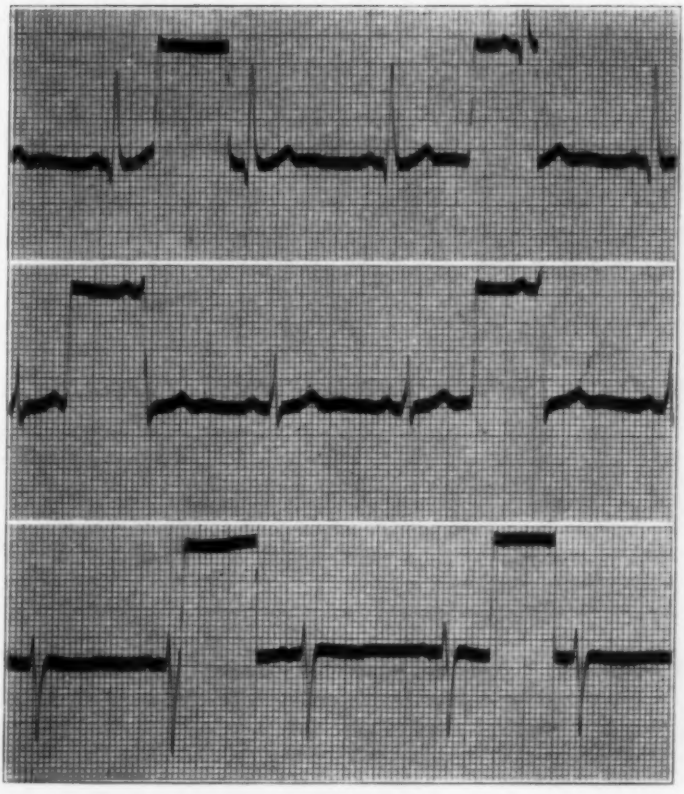

Fig. 5.—This electrocardiogram was taken with immersion nonpolarizable electrodes after rubbing the skin with salt solution. The resistance of Lead 1 is 2,500; Lead 2, 2,500, and Lead 3, 2,500 ohms.

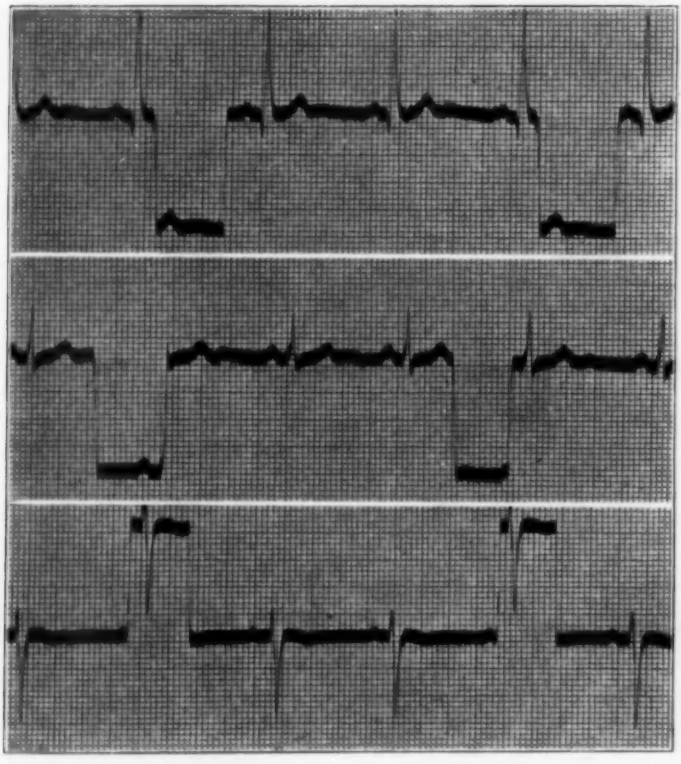

Fig. 6.—This electrocardiogram was taken with plate electrodes after rubbing the skin with salt solution. The resistance of Lead 1 is 2,300; of Lead 2, 2,300; of Lead 3, 1,400 ohms.

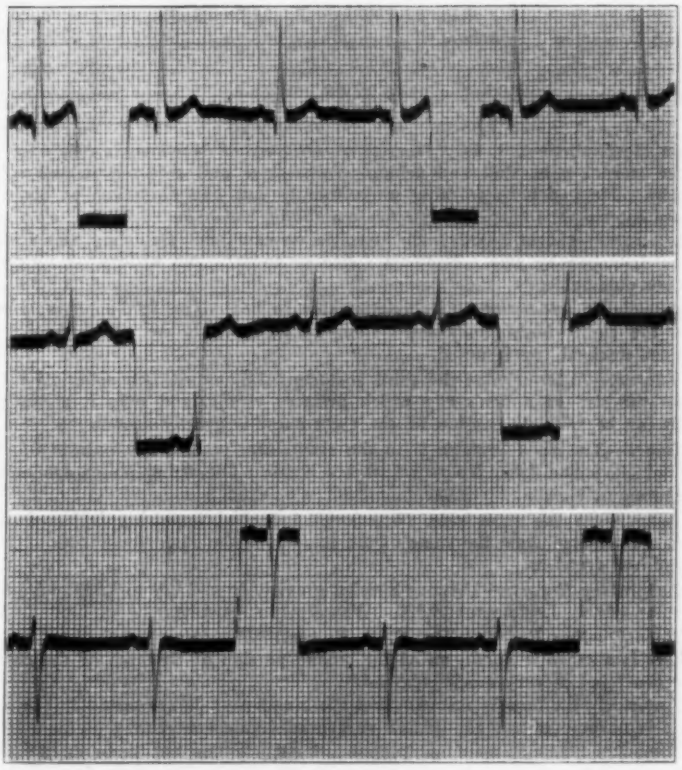

Fig. 7.—This electrocardiogram was taken with German silver electrodes after rubbing the skin with salt solution. The resistance of Lead 1 is 1,400; of Lead 2, 1,400; of Lead 3, 1,400 ohms.

FROZEN SECTIONS FROM A CASE OF PROTRUDING ANEURYSM OF THE ARCH OF THE AORTA

GEORGE WILLIAM NORRIS, A.B., M.D.

AND

GEORGE FETTEROLF, M.D., Sc.D.

PHILADELPHIA

REPORT OF CASE

History.—A negro laborer, aged 39 years, was admitted to the Pennsylvania Hospital, Sept. 1, 1919 (service of Dr. Arthur Newlin), complaining of upper sternal pain, dull and aching in character and referred to the right scapula. He had been working intermittently, but had not taken to his bed before entering the hospital. For five months he had been troubled with cough, expectoration (never bloody) and dyspnea on exertion. His sleep was sometimes disturbed by pain. Nycturia (once or twice). No history of venereal disease obtainable.

Physical Examination.—Lies comfortably in bed. The left pupil is larger than the right; both pupils seem sluggish. The teeth are poorly kept. A distinct tracheal tug is noted.

Thorax.—Anteriorly, at the third costosternal juncture, is a spherical mass about the size of a hen's egg which pulsates visibly, and over it a systolic thrill can be felt. Expansion of the right chest is greatly diminished.

Heart.—The apex impulse is in the sixth interspace, 3 cm. outside of the left midclavicular line. No murmurs are heard. The radial pulses are equal, synchronous, slow and regular.

Lungs.—On the right side, posteriorly, bronchial breathing is heard and dulness is elicited from the spine of the scapula to the base, also egophony and diminished fremitus.

Laboratory Reports.—Urine: Specific gravity, from 1.020 to 1.030; no albumin; no casts. Leukocytes: 9,520. Wassermann: positive.

Roentgen-Ray Report.—(Dr. D. R. Bowen.) Fluoroscope and plate study: "There is a saccular aneurysm of the second portion of the arch of the aorta. The heart is not very much enlarged."

Anteriorly the lung is resonant and the breath sounds are distant. The left chest is normal, except for dilated superficial cutaneous veins.

Sept. 22, 1919.—Patient has been irrational for the last three days. The tumor mass has increased, now measuring 11 cm. in diameter. Respirations are difficult. Wiring of the aneurysm was discussed with Dr. J. H. Gibbon, but decided against.

September 23.—The patient's condition has gradually grown worse. Dyspnea, orthopnea and chest pain have necessitated the use of morphin. At 1 a. m. respirations became very laborious. Edema of the lungs was developing which was not checked by atropin or phlebotomy. Death at 2 a. m., apparently from pulmonary edema.

Having obtained possession of the (unclaimed) body through the courtesy of Dr. Addinell Hewson of the State Anatomical Board, the body was formalinized, frozen and cut in the usual manner.

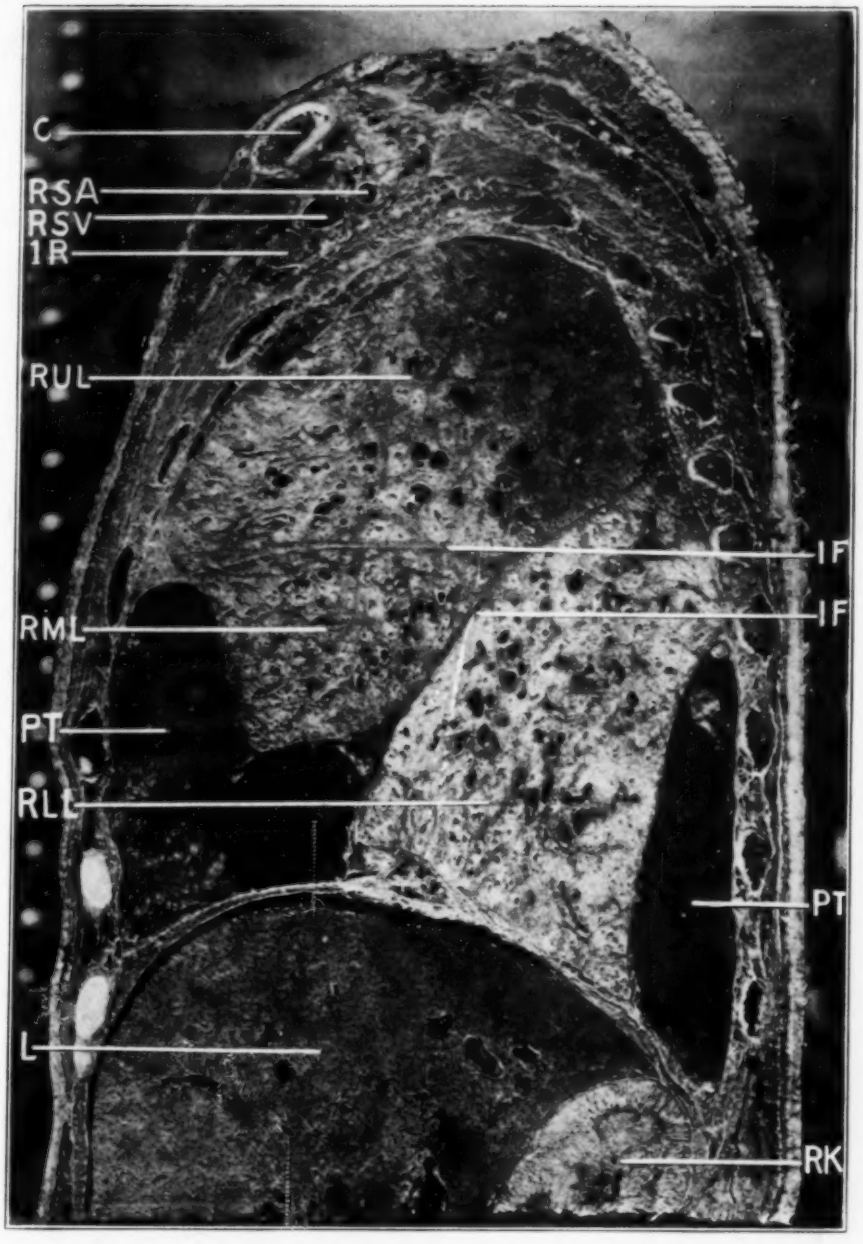

Fig. 1.—C, clavicle; RSA, right subclavian artery; RSV, right subclavian vein; 1R, first rib; RUL, right upper lobe; RML, right middle lobe; PT, PT, pleural transudate; RLL, right lower lobe; L, liver; RK, right kidney; IF, IF, interlobar fissures.

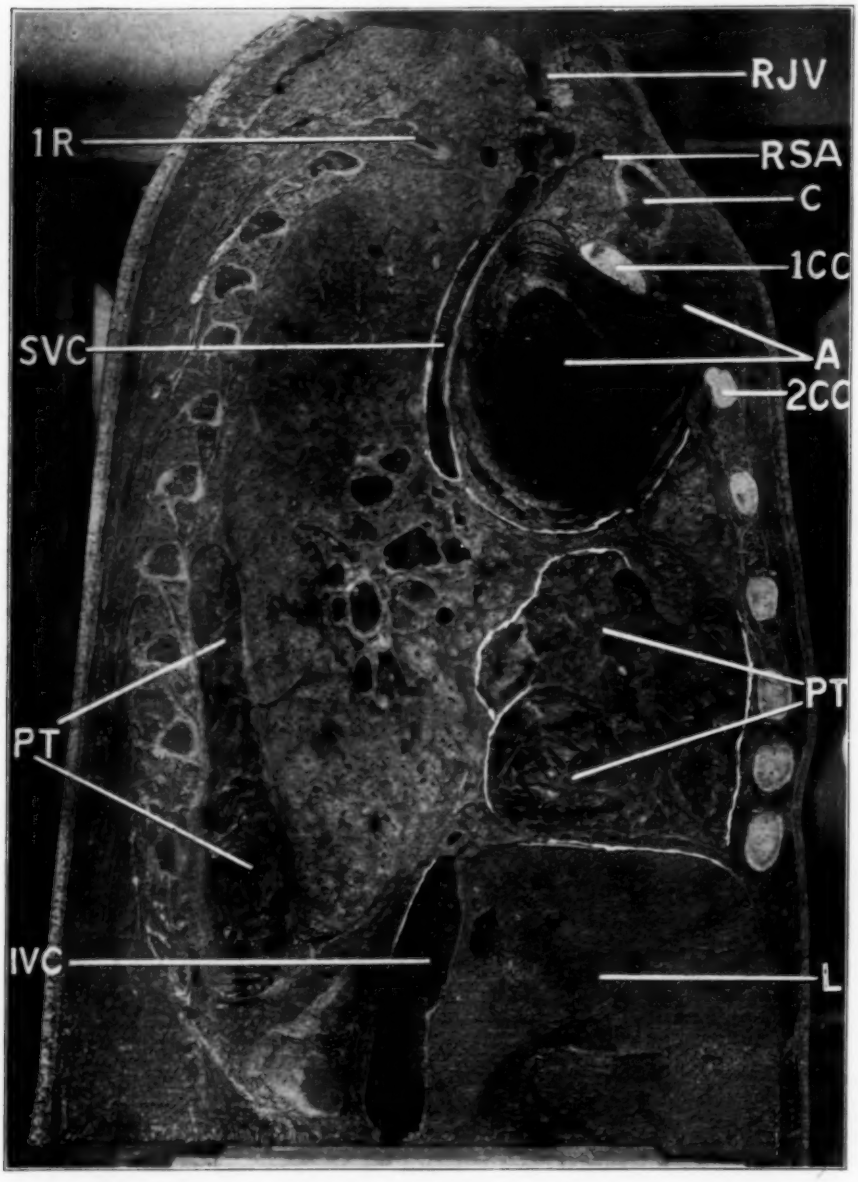

Fig. 2.—1R, first rib; SVC, superior vena cava; PT, PT, pleural transudate; IVC, inferior vena cava; L, liver; 2CC, 1CC, second and first costal cartilages; A, aneurysm; C, clavicle; RSA, right subclavian artery; RJV, right internal jugular vein.

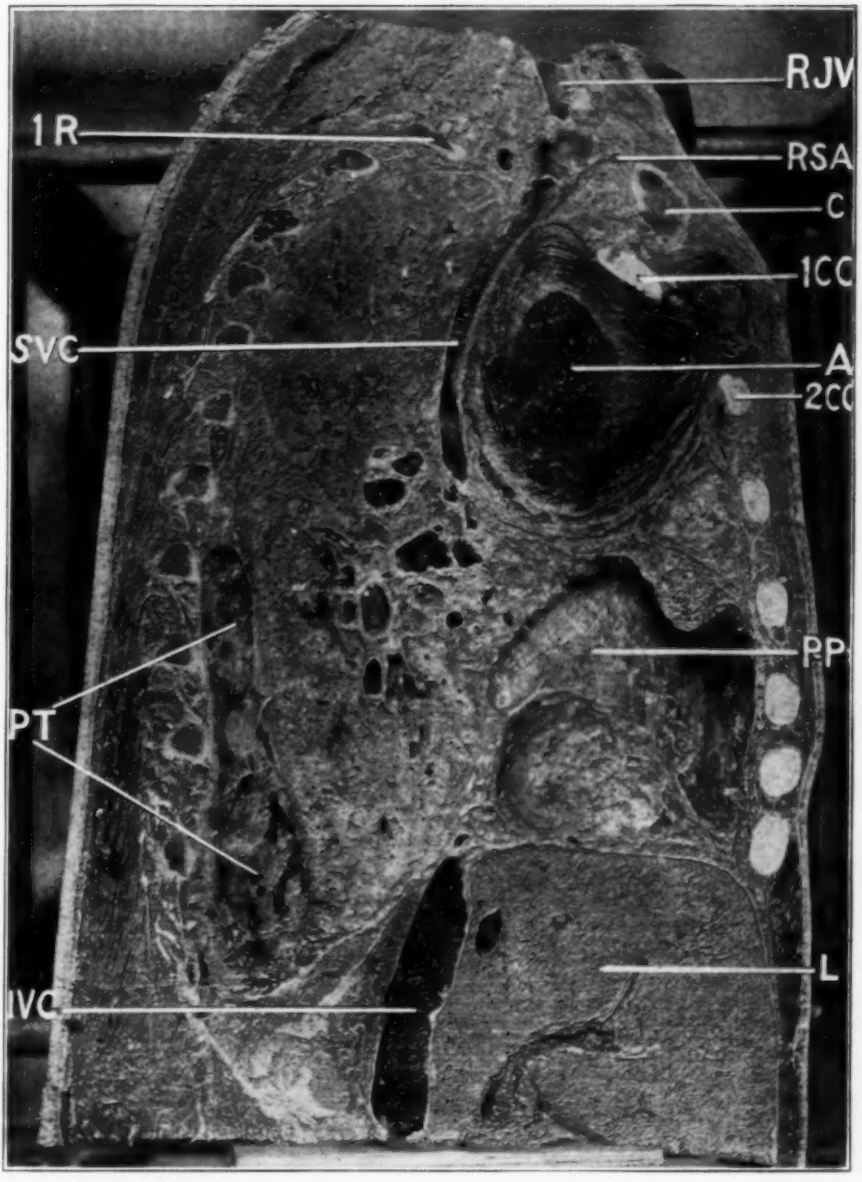

Fig. 3.—1R, first rib; SVC, superior vena cava; PT, pleural transudates; IVC, inferior vena cava; L, liver; P-P, pleuro-pericardium; 2CC, 1CC, second and first costal cartilages; A, aneurysm; C, clavicle; RSA, right subclavian artery; RJV, right interior jugular vein.

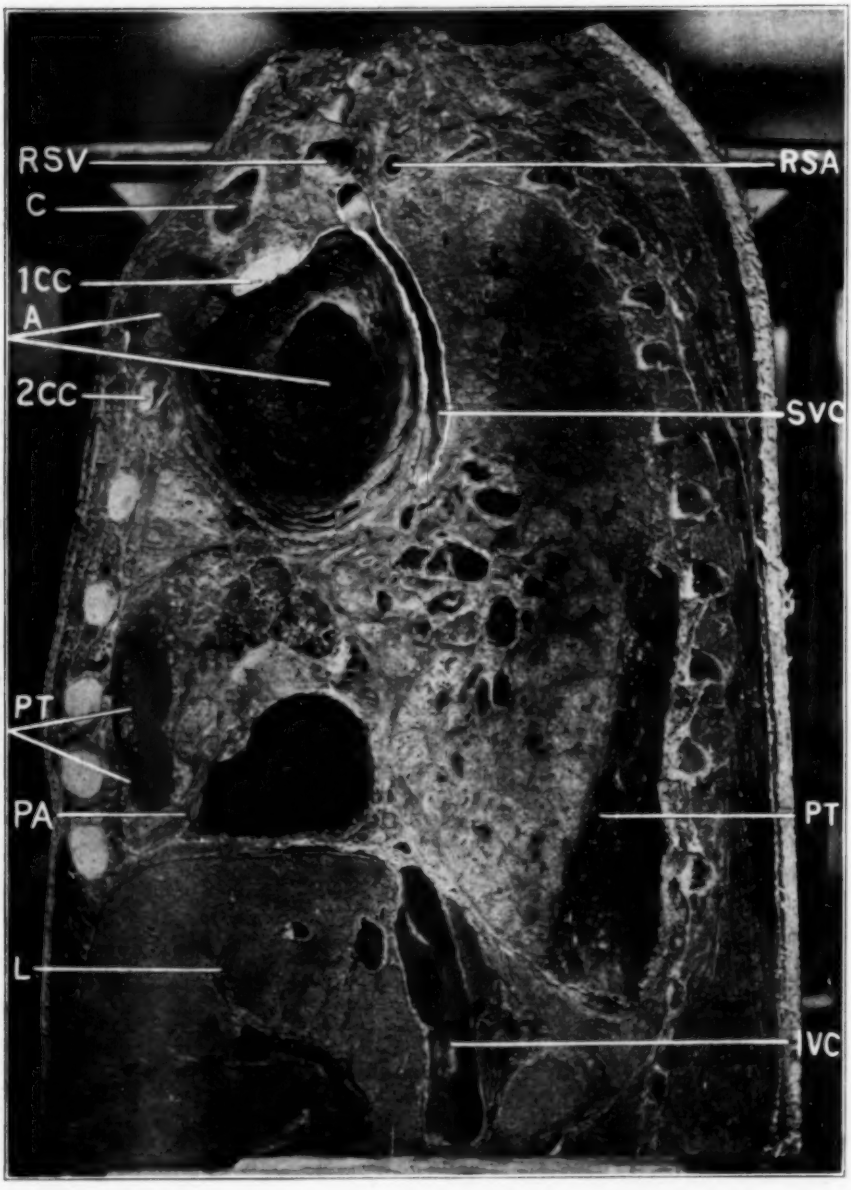

Fig. 4.—RSV, right subclavian vein; C, clavicle; 1CC, 2CC, first and second costal cartilages; A, aneurysm; PT, PT, pleural transudate; PA, pleural adhesion; L, liver; IVC, inferior vena cava; SVC, superior vena cava; RSA, right subclavian artery.

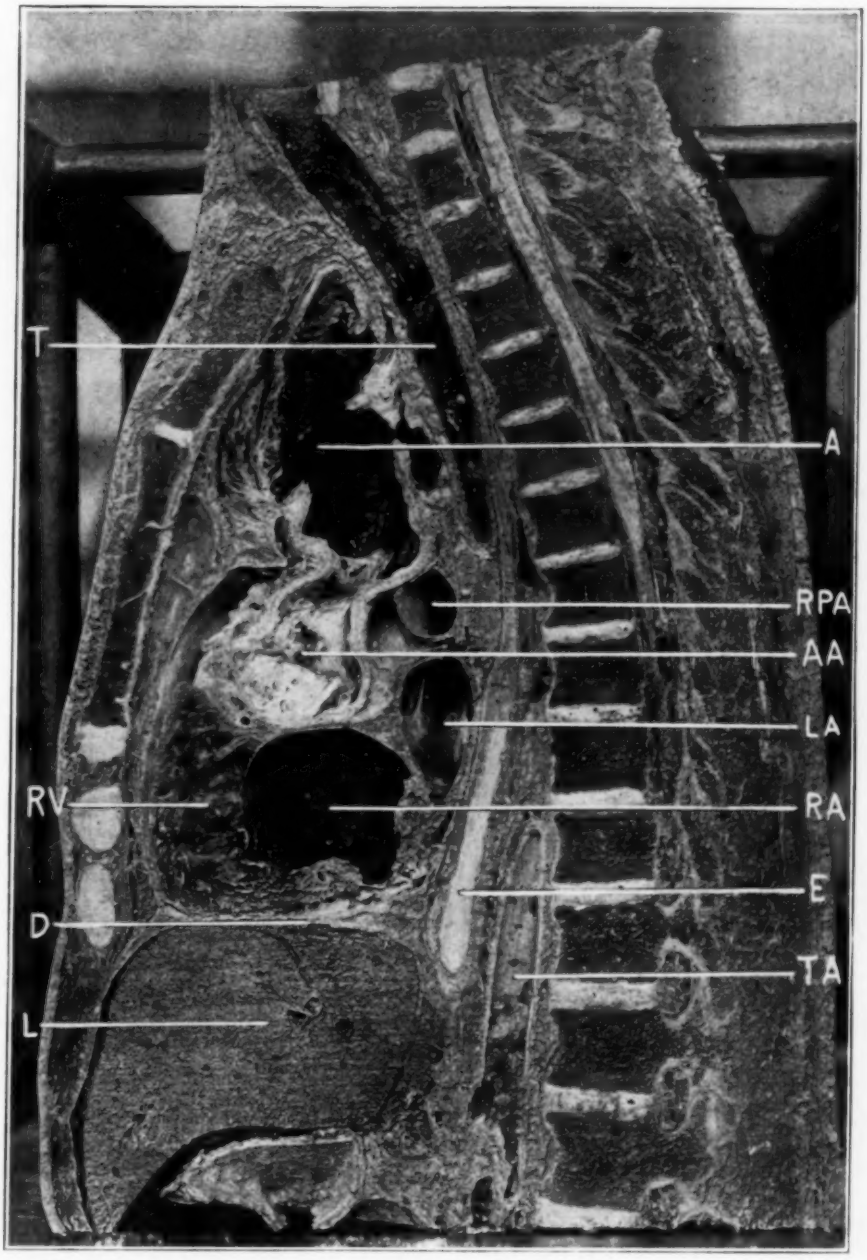

Fig. 5.—T, trachea; RV, right ventricle; D, diaphragm; L, liver; TA, thoracic aorta; E, esophagus; RA, right auricle; LA, left auricle; AA, ascending part of aortic arch; RPA, right pulmonary artery; A, aneurysm.

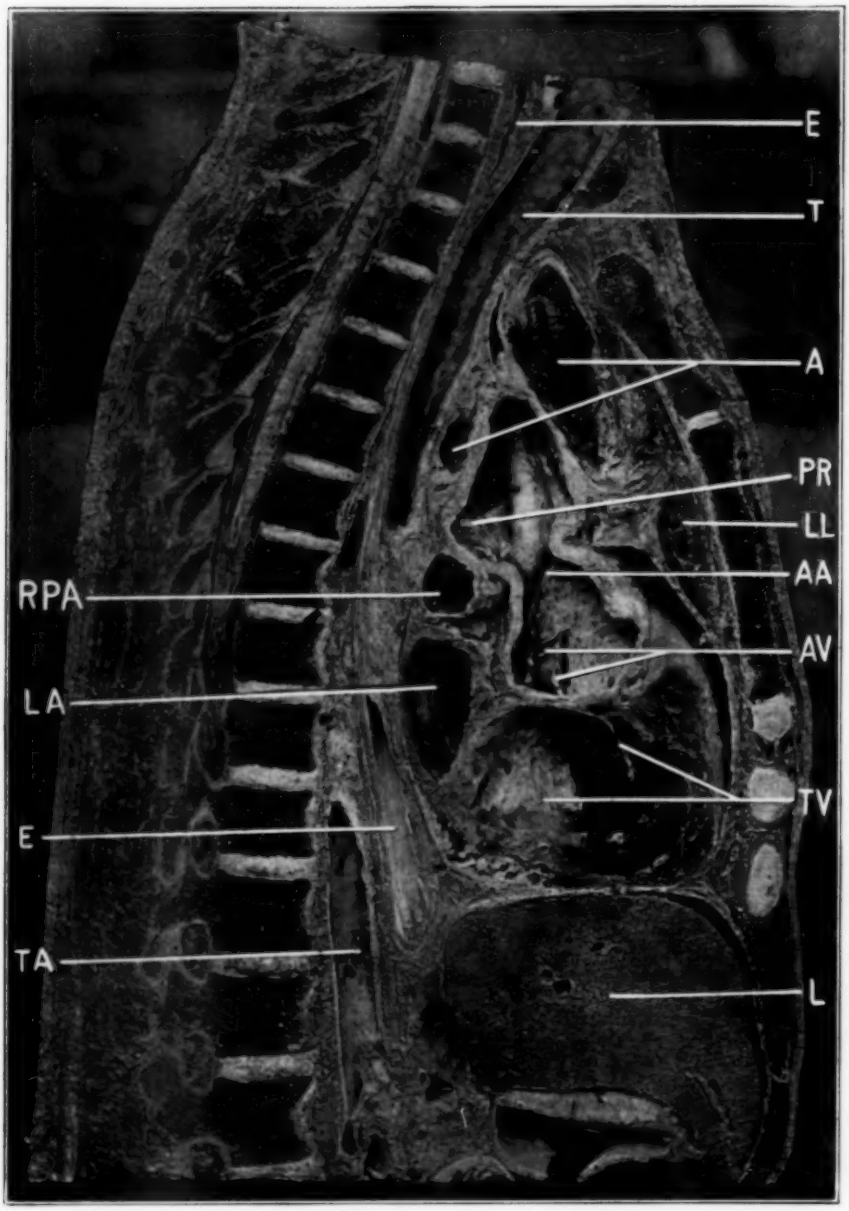

Fig. 6.—RPA, right pulmonary artery; LA, left auricle; E, esophagus; TA, thoracic aorta; L, liver; TV, tricuspid leaflets; AV, aortic leaflets; AA, ascending portion of aortic arch; LL, small portion of left lung; PR, probe in opening between aneurysm and trachea; A, aneurysm; T, trachea.

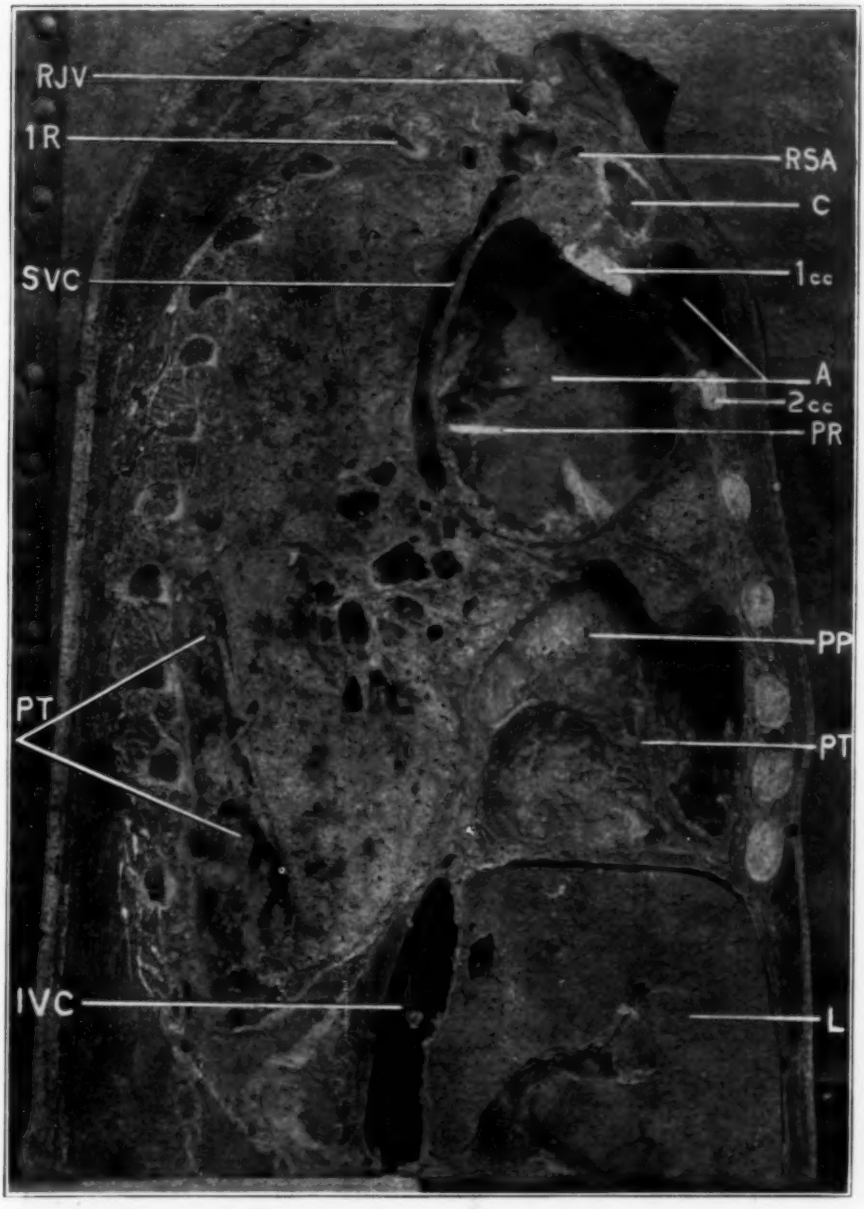

Fig. 7.—RJV, right internal jugular vein; 1R, first rib; SVC, superior vena cava; PT, PT, pleural transudate; IVC, inferior vena cava; L, liver; P-P, pleuro-pericardium; PR, probe in opening between trachea and aneurysm; 2CC, 1CC, second and first costal cartilages; A, aneurysm; C, clavicle; RSA, right subclavian artery.

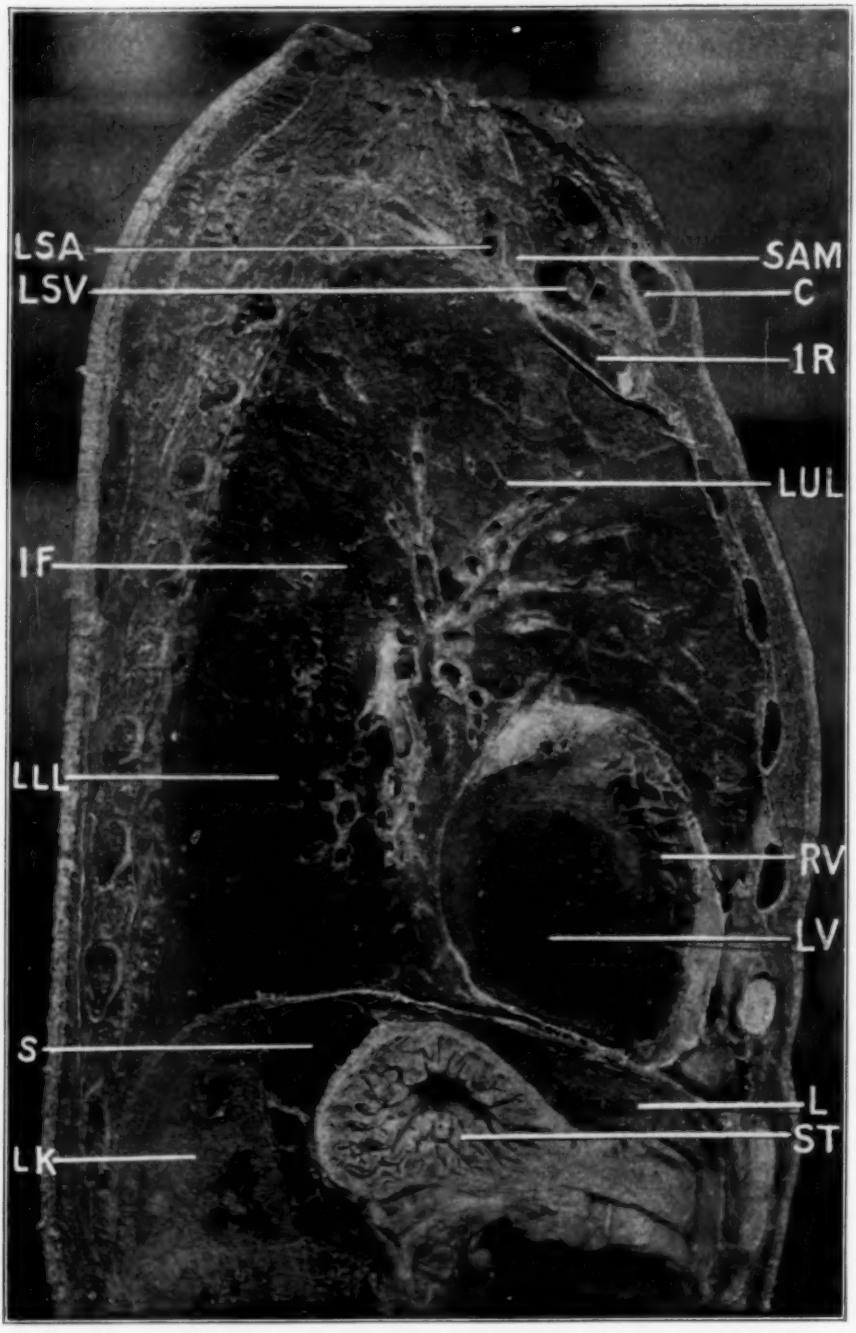

Fig. 8.—LSA, left subclavian artery; LSV, left subclavian vein; IF, interlobar fissure; LLL, left lower lobe; S, spleen; LK, left kidney; ST, stomach; L, liver; LV, left ventricle; RV, right ventricle; LUL, left upper lobe; 1R, first rib; C, clavicle; SAM, scalenus anticus muscle.

DISCUSSION

Figure 1, which was cut approximately through the middle of the right lung, shows two pleural vomicae caused by loculation of an effusion. The lower pulmonary lobe is densely and firmly adherent to the central portion of the diaphragm, and the interlobar fissures show firm fibrous adhesions. The lung itself, in addition to atelectasis and compression of its lower portions, shows a widespread fibrosis which is strikingly in contrast to the left lung shown in Figure 8.

Inasmuch as the past medical history failed to indicate any antecedent pulmonary disease, such as pneumonia or pleurisy, one is forced to conclude that the fibrosis resulted from long standing hydrothorax, despite the fact that the general appearance would suggest an antecedent pneumonia complicated by an unrecognized empyema.

Figure 2, cut nearer to the median line, shows the pleural vomicae still filled with frozen serum (ice crystals) and a section of the aneurysm. The latter is filled with a laminated clot and protrudes between the first and second ribs. The superior vena cava immediately behind it is seriously encroached on, in contrast to the inferior vena cava, and explains the venous engorgement of the neck and head.

Figure 3 shows the same section, after the removal of the frozen serum from the pleural vomicae.

Figure 4 shows the next section, in which a dense, fibrous adhesion extends from the anterior portion of the lower lobe to the diaphragm, causing still another loculation of the pleural cavity. The liver immediately underlying this region is noticeably flattened.

Figure 5, the section of which passes through the middle of the sternum and the vertebral column shows the sacular character of the aneurysm. The ascending aorta is aneurysmal. Above this it retains approximately its normal size, to dilate again and to a much greater degree. It is partially filled with laminated clot and fresh blood clot. The trachea behind the aneurysm is filled with clot.

Figure 6, the other half of the median section (Fig. 5) well shows the first sacculation of the aneurysm, immediately above the aortic leaflets, as well as a second and third sacculation. The latter, the smallest, lies immediately in front of the trachea at the level at which the rupture occurred (Fig. 7).

Unlike a somewhat similar case previously reported,[1] the perforation did not occur at a point in the direct line of the blood stream. The trachea and the left auricle are both noticeably encroached on by the aneurysm. It will be noted that the sternum is intact, the aneurysm having protruded between the ribs.

1. Tr. A. Am. Phys. **32**:420, 1917.

Figure 7 (Fig. 3 with the aneurysmal clot removed) shows a match stick inserted into the perforation which opened into the trachea, which promptly became filled with blood as shown in Figure 5.

Figure 8, a section through the left lung shows a normal lung somewhat congested at the base. This cut should be compared with Figure 1, which shows extensive pulmonary fibrosis.

It is generally believed that in only about 50 per cent. of all cases of thoracic aneurysm does death occur by rupture.

This patient appeared to die from pressure and from exhaustion. Although no blood was coughed up, the trachea was filled with it, death occurring from suffocation due to rupture. It seems not unlikely that rupture may account for more deaths than purely clinical records, without a postmortem, would lead us to believe.

1820 South Rittenhouse Square.

RESULTS OF ANTEMORTEM LUNG PUNCTURES IN LOBAR PNEUMONIA

THEIR BEARING ON THE MECHANISM OF CRISIS *

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Typing of pneumococci in the sputum of patients suffering from lobar pneumonia was begun in this laboratory in March, 1919. In order to corroborate the results, lung punctures were performed at the same time and the organisms thus obtained were checked up with those in the sputum. As time went on, aside from this corroborative value, the results of the lung punctures appeared to us to have an interest from quite a different point of view, namely, as throwing some light on the nature of the mechanism of crisis. It is from this point of view that we are presenting our results. In so doing, we fully realize that the work itself is not original; nevertheless, it seems to be of some value both as confirming the results of our predecessors and as a stimulus possibly to others to attempt similar work. There seems to be some necessity for this, as there appears to be a general impression among clinicians that lung puncture is a hazardous procedure, apt to produce such complications as empyema; in answer to such objections and to allay such fears, we can say that we never have seen any unfavorable results, such as empyema, hemorrhage, etc., following lung puncture. It is only fair to state that one patient did die suddenly, but in this instance the chest was being tapped for suspected fluid, and a lung puncture was not being done intentionally. Such an unfortunate accident is, of course, to be encountered in any large series of chest taps, and, therefore, should not be considered as an objection to lung puncture.

A review of the literature shows that antemortem lung puncture has been a relatively infrequent procedure, though first done in 1883 by Leyden.

Leyden [1] found organisms in one out of three cases in which he performed lung puncture. That patient died.

Gunther,[2] suspecting pus, was rewarded by finding diplococci in the exudate withdrawn by puncture of the lung.

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* From the Pneumonia Service and Pathological Laboratory, Boston City Hospital, Boston.

* Presented May, 1920, before the American Society for Clinical Investigation.

1. Leyden, I.: Deutsch. med. Wchnschr. 9:52, 1883.
2. Günther: Deutsch. med. Wchnschr. 9:52, 1883.

Talamon [3] performed postmortem lung punctures in nine cases, finding pneumococci in smears of the exudate in eight.

Patella [4] performed a series of punctures before and after crisis. In all those done before crisis the exudate contained a large number of pneumococci. Punctures done after crisis gave inconstant results, but in the exudate withdrawn by puncture during the hours immediately following defervescence, this author was able to recover viable pneumococci.

Tchistovitch [5] practiced punctures, inoculated the exudate into rabbits and mice and was able to convince himself that even after crisis one can withdraw from the lungs by puncture an exudate containing not only viable but virulent diplococci. Therefore, he said, crisis cannot depend on the destruction of all the diplococci in the lung. He went on to say that crisis occurs when toxin formation by the pneumococci ceases, due to their being taken up by the phagocytes, and kidney elimination increases.

Rosenow [6] made forty-eight punctures in twenty-seven cases to study the bacteriologic and cellular content of the lung exudate more especially from a point of view of phagocytosis. Of the forty-eight, twenty-six yielded growths of pneumococci in pure culture; twenty-two were sterile. Positive punctures were obtained at all stages of the disease, but the percentage of positive results was much higher in the early stages of favorable cases and throughout the course in those patients that died. Early in the course of fatal and nonfatal cases the number of positive results was the same; in favorable cases the number gradually diminished as crisis was reached. Only six out of twenty punctures during or after crisis yielded organisms, and only a few colonies in each, while in patients who died the number became progressively greater as death approached.

The technic of lung puncture is very simple. In selecting the point to puncture the area of maximum solidification as determined by physical signs is chosen. After cleansing the skin with alcohol and iodin, local anesthesia is produced with 0.5 per cent. procain. A No. 16 needle is then introduced between the ribs, through the pleura and into the lung tissue to the depth of about one inch. Negative pressure is then maintained with a 10 c.c. or 20 c.c. syringe and the needle slowly withdrawn. In this way a few drops of bloody fluid are obtained and may then be cultured in broth and on blood agar and a film made on a slide.

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3. Talamon, M. le D.: Bull. Soc. Anat. de Par. **63**:475, 1883; also Progrès méd. **11**:281, 301, 1883.
4. Patella, V.: Attr. d. R. Academia di Roma, **4**: 1888.
5. Tchistovitch, N.: Ann. de L'Inst. Pasteur **18**:304, 1904.
6. Rosenow, E. C.: J. Infect. Dis. **8**:500, 1911.

For the sake of brevity, we designate lung punctures yielding living pathogenic organisms which were cultivated and studied as "positive lung punctures," those which yield sterile cultures as "negative lung punctures." From a direct smear of the exudate little of value was learned; usually, however, a smear which failed to show organisms was associated with negative cultures also.

In Table 1, where the positive and negative punctures are charted according to the day of disease on which they were performed, it will be noticed that the two series run almost parallel in this respect.

TABLE 1.—Record of Positive and Negative Punctures

	Day of Disease															Total	Mortality
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
Positive lung punctures.....	0	1	5	4	7	4	5	7	5	1	2	0	0	0	1	42	42.00%
Negative lung punctures....	..	1	3	4	3	5	5	3	1	2	1	1	0	0	2	31	6.45%

Table 2 takes into account the organism obtained from punctures and also shows which of the patients died (those indicated by the lower line of figures in each square). The abnormally high mortality for the individual type and for this whole group is quite striking (six of the Type 1 patients who recovered were given treatment with antipneumococcus serum). It is also interesting to note that the mortality in the cases yielding positive punctures before the sixth day is 23.5 per cent., whereas in those positive on and after the sixth day the mortality is 56 per cent.

Table 2 also illustrates the negative punctures, the bacteriologic grouping being obtained from the sputum examination. Only two of these patients died — one five days and the second three days after the puncture — each having an extension of the pulmonary lesion into a fresh lobe. An adequate explanation for the occurrence of these two negative punctures is not at hand unless one admits the possibility of local recovery in the face of an active and eventually fatal process elsewhere. Further evidence that this may occur is seen at necropsy in cases in which one lobe is undergoing resolution while another is in the stage of red or gray hepatization.

A point which is not illustrated in these tables is that positive lung punctures were obtained on every day of the disease including the period of critical fall of the temperature and twice after the temperature had reached normal. On the other hand, one negative puncture four days before crisis, two negative punctures three days before crisis and six negative punctures two days before crisis show quite clearly that, in cases which are going on to recovery, the organisms may diminish to a great extent some time before crisis occurs. If, as has been claimed, a sudden destruction of the organisms takes place at the

TABLE 2.—Day of Disease on which Puncture Was Performed, and Mortality

Positive Lung Punctures

	Day of Disease															Well	Dead	Total	Per Cent. of Cases	Mortality, Per Cent.
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15					
Pneumococcus Type I	..	..	3	2	4	2	1 1	1	1 1	..	.. 1	..	..	..	.. 1	14	4	18	43	22
Pneumococcus Type II	..	.. 1	.. 1	1	1	.. 2	.. 1	2 1	.. 1	..	..	..	..	..	..	4	7	11	26	64
Pneumococcus Type III	..	..	..	..	.. 1	..	.. 1	..	1	.. 1	.. 1	..	..	..	..	1	4	5	12	80
Pneumococcus Type IV	..	..	1	1	.. 1	..	.. 1	1 1	1	.. 0	..	..	..	..	..	4	3	7	17	43
Pneumococcus Type (?)	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	1	..	1	2	0
Well	..	..	4	4	5	2	1	5	3	..	..	..	..	..	..	24				
Died	..	1	1	0	2	2 1	4	2	2	1	2	..	..	..	1		18			
Total	..	1	5	4	7	4	5	7	5	1	2	..	..	..	1	..	..	42	100	43

Negative Lung Punctures

	Day of Disease															Well	Dead	Total	Per Cent. of Cases	Per Cent. of Cases	Mortality, Per Cent.
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15						
Pneumococcus Type I	..	1	1	..	1	1	1	2	..	..	..	..	..	..	..	7		7	29	22	0
Pneumococcus Type II	..	..	..	1	..	..	3	..	..	2	..	..	..	..	..	6		6	25	19	0
Pneumococcus Type III	..	..	..	1 1	..	..	..	1	..	..	..	1	..	..	1	4	1	5	21	17	20
Pneumococcus Type IV	..	..	..	..	..	2 1	..	..	1	..	1	..	..	..	1	5	1	6	25	19	16
Nonhemolytic streptococci	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	1		1		3	0
Unknown	..	..	1	1	2	1	1	..	..	..	..	..	..	..	..	6		6		19	0
Well	..	1	3	3	3	4	5	3	1	2	1	1	..	..	2	29					
Died	..	..	..	1	..	1	..	..	..	..	..	..	..	..	..		2				
Total	..	1	3	4	3	5	5	3	1	2	1	1	..	..	2	..	..	31	..	100	6.5

The lower line of figures in each square represent the deaths.

time of crisis, positive lung punctures should be obtained with great regularity up to a short time before crisis in every case and not after crisis.

The fact which interests us most is that the number of organisms may begin to diminish fairly early in the disease, even three or four days before crisis in favorable cases, but fails to diminish throughout the course of cases which end in death. According to our conception, the organisms may be all killed twenty-four or more hours before crisis, or may continue to exist twenty-four hours after crisis. Thus the two mechanisms, i. e., bactericidal and detoxifying, are somewhat independent of one another, the second taking place at a given time after the first has gained control of the infection. (A pseudocrisis may even occur before the infection is properly under control.) As the organisms cease, then, to dominate the situation by their active growth in the lung, the detoxifying mechanism has a chance to become efficacious. Detoxication may occur in three ways: (1) By a cessation in toxin production; (2) by an increase in the antitoxic bodies to a point sufficient to neutralize the toxin as fast as it is produced, and (3) by a conversion of the toxic into nontoxic products. That the two mechanisms mentioned above — bactericidal and detoxifying — exist is by no means a new thought. But we offer this argument as a further point in favor of their existence.

The literature, since the discovery of the pneumococcus, has been filled with accounts of futile attempts to isolate pneumotoxins and produce antitoxins. G. and F. Klemperer,[7] as early as 1891, described the satisfactory use of serum in curing rabbits infected with pneumococcus and ascribed the results to the antitoxic properties of the serum. It is unnecessary here to review the entire literature on this subject. Suffice it to say that the consensus of opinion today [8] is that the pneumococcus contains preformed anaphylatoxin which is also hemolytic for red blood cells, and which is mildly antigenic. The antiserum formed by this anaphylatoxin (proteotoxin) cannot be used to produce passive immunity and is thought of little practical importance in the mechanism of recovery from pneumonia. The humoral and cellular activities described by various workers were well reviewed by Wadsworth [9] in 1912. The important agencies which had been shown to exist were serum bacteriolysis (slight), bactericidal property of whole blood (slight), increase in phagocytosis by the opsonins and bacteriotropins; and agglutinins and precipitins. Since that time anti-

7. Klemperer, G. and F.: Berlin klin. Wchnschr. **28**:833, 869, 1891. (Therap. Gaz., Aug.)

8. Cole, R.: New York M. J. **101**:1, 59, 1915. Rosenow, E. C.: J. Infect. Dis. **9**:190, 1911. Cole, R.: J. Exper. Med. **20**:346, 1914. Clough, P. W.: Johns Hopkins Hosp. Bull. **26**:37, 1915. Weiss, C., and Kolmer: J. Infect. Dis. **22**: 469, 1918. Solis-Cohen, Weiss and Kolmer: J. Infect. Dis. **22**:476, 1918.

9. Wadsworth, A. B.: J. Exper. Med. **16**:54, 1912.

blastic immunity [10] has been demonstrated and discussed, sensitizing antibodies present in the blood before but not after crisis [11] have been claimed and the action of various chemicals in conjunction with immune serum has been demonstrated.[12] Studies on the action of immune serum [13] administered to animals in which a pneumococcus septicemia is produced have led to the belief that the activities of the serum are due to agglutination of the organisms and bacteriotropic power of the serum. More recently the mechanism of the effect of whole blood [14] of animals naturally immune or actively and passively immunized has been shown to depend on the opsonization of the pneumococci by the immune serum and the phagocytosis of the organisms by the polynuclear leukocytes. But none of these factors offers a satisfactory explanation of the phenomenon of crisis.

From a somewhat different point of view the effect of the acid produced in the course of normal growth of the pneumococcus [15] has been offered as cause for the death of the organism and activating of the proteolytic enzymes.[16] Following the lead of Vaughan,[17] a great deal of most suggestive work on the toxic split products of the various proteins has been done. Whether these toxic products are formed by partial splitting of the pneumococcus protein, the pneumonic exudate proteins, or the serum proteins has not been cleared up, but Petersen and Short [18] favor the lung exudate as the source. Jobling, Petersen and Eggstein [19] suggest, however, that in the production of crisis the rapid death of a large number of pneumococci takes place, thus making possible the rapid and complete action of the ferment unhindered by antiferment and toxic by-products of bacterial growth. The results obtained by these and other workers studying pneumonic blood and pneumonic exudate from a biochemical point of view, further strengthen the possibility that the toxemia in pneumonia is due to the constituents of the lung exudate, and that detoxication is due to an increase or alteration in ferment action whereby these toxic products

10. Dochez, A. R., and Avery, O. T.: J. Exper. Med. **23**:61, 1916.
11. Weil, R., and Torrey, J. C.: J. Exper. Med. **23**:1, 1916.
12. Lamar, R. V.: J. Exper. Med. **26**: **27**: 1911. Kolmer, J. A., and Steinfield, E.: J. Infect. Dis. **22**:492, 1918.
13. Bull, C. G.: J. Exper. Med. **22**:457, 484, 1915.
14. Bull, C. G.: J. Exper. Med. **31**:233 (March) 1920.
15. Lord, F. T., and Nye, R. N.: J. Exper. Med. **30**:389 (Oct.) 1919. Avery, O. T., and Cullen, G. E.: J. Exper. Med. **30**:359 (Oct.) 1919.
16. Lord, F. T.: J. Exper. Med. **30**:379 (Oct.) 1919. Lord, F. T.: J. A. M. A. **73**:1364 (May 10) 1919.
17. Vaughan, V. C., V. C. Jr., and J. W.: Protein Split Products, Philadelphia, Lea and Febiger, 1913.
18. Petersen, W., and Short, C. A.: J. Infect. Dis. **22**:147, 1919.
19. Jobling, J. W.; Petersen, W., and Eggstein, A. A.: J. Exper. Med. **22**: 568, 1915.

are rendered nontoxic. Weiss assumes a sudden destruction of large numbers of organisms at a time when the greatest amount of pneumotoxin and hence of toxin albumoses, of proteoses, etc., exists. He speaks of this as the period of crisis. We do not believe that this sudden destruction of organisms plays a part in crisis. We believe that a second mechanism, namely, that which serves to neutralize or destroy the toxic substances, acts, to a large extent, independently. The rate of development of the antibacterial bodies, on the one hand, and the antitoxic mechanism on the other, may occur simultaneously or not, according to factors which influence their separate production differently. Thus an early destruction of the organisms would leave little to be undertaken by the antitoxic mechanism whereas a late checking of the bacterial growth would require a much more active antitoxic effort on the part of the patient. Blake and Cecil,[20] from observations of a somewhat similar nature during the course of their work on experimental pneumonia in monkeys, suggest the possibility of a dual mechanism: one humoral, the other local and possibly biochemical in nature. Our conception differed slightly from theirs in that we believe the humoral reaction has a definite influence on the local pulmonary lesion and thus makes possible the fulfillment of the second mechanism, which is of a detoxifying nature and may or may not be entirely local.

There are certain facts that make the presence of a toxin in lobar pneumonia seem possible. In lobar pneumonia we have a local process with general symptoms and with lesions in the important organs. Microscopically, in cases of lobar pneumonia we find definite necroses in the liver, suprarenal, pancreas and kidney; lesions definitely toxic in origin, not due to the actual presence of the organisms, but caused by a toxin in the general circulation in every way analogous in type to the lesions seen in diphtheria. We know that this type of lesion can be produced by a bacterial exotoxin, but whether it can also be caused by a so-called proteotoxin (anaphylatoxin) or by toxic split products derived from the host's proteins has yet to be definitely demonstrated.

There are many explanations for the failure to demonstrate a toxin in the blood or lung exudate of a patient suffering from pneumonia; as for instance:

1. That the toxin is fixed by the tissues (or when standing in vitro combines with antibodies).

2. That there is such a thing as "species specificity" in toxins; i. e., that the toxin produced in the lung of a human being is toxic only for man, since it is formed from the proteins peculiar to man.

Meanwhile, the nature of the toxin remains undetermined. We know that there is no demonstrable soluble pneumotoxin. We know

20. Blake, F. G., and Cecil, R. L.: J Exper. Med. **31**:460, 480 (April) 1920.

that the proteotoxin of the pneumococcus plays but a small part in causing the symptoms of toxemia, and we know that similar symptoms are caused by autolysis of aseptic tissue. This lends plausibility to the suggestions of Cole [21] and Jobling, Petersen, Eggstein and others, that the higher split proteins from the lung exudate are responsible for the toxemia in pneumonia. The possibility of a "species-specific" toxin formed by the growth of the pneumococcus in the lung exudate must still be considered. If a toxin exists, the production and importance of an antitoxin are pressing problems.

A solution to these questions is being sought at present but too little progress has as yet been made to justify a report.

SUMMARY

Study of lung punctures performed at different stages of lobar pneumonia, shows that the death of the organisms in the lung does not occur, in every case, at the time of crisis, but may occur several days before or after crisis. This leads to the belief that the anti-bacterial forces may proceed at one rate of speed while the detoxifying mechanism is going on at a different rate. The nature of the toxic substance in lobar pneumonia is imperfectly understood but the demonstration of toxic split proteins does not finally rule out the possibility of a true toxin-antitoxin reaction.

CONCLUSIONS

The death of the pneumococcus is not the factor which causes or initiates crisis.

The antibacterial and detoxifying mechanisms act, in a measure, independently.

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21. Cole, R.: Arch. Int. Med. **14**:56 (July) 1914.